ATTACHMENT X
WILD RICE STUDIES

X1 ***The Effect of Heavy Metals on the Early Growth of Wild Rice. Lee, Peter. Lakehead University. Ontario, Canada. 1996.***

X2 ***The Ecology of "Wild" Wild Rice (Zizania Palustris var. Palustris) in the Kakagon Sloughs, a Riverine Wetland on Lake Superior. Meeker, J. Proceedings of the Wild Rice Research and Management Conference. Carlton, Minnesota. July 7 – 8, 1999.***

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Dr. P.F. Lee

August 30, 1996

Letter of Transmittal

Ms. Anne McCammond Soltis
Great Lakes Indian Fish and Wildlife Commission
Odanah, Wisconsin 54861
U.S.A.

Dear Anne:

I am pleased to attach a copy of the report entitled "The Effect of Heavy Metals on the Early Growth of Wild Rice" which will complete the contract between your agency and Lakehead University.

As outlined in our proposal, we examined the effects of Al, Cu, Cd, Pb and Hg on the early development of wild rice. We determined the concentrations of these elements in the solution and tissue of the exposed samples. Finally we compared our results with wild rice to the effect on common duckweed which has been used for similar types of experiments.

Our results were quite striking. All elements examined had pronounced adverse effects on wild rice. The most noticeable changes in the affected plants were reduced leaf and root area. Chlorosis of leaves and roots was also present. Mortality of the seedlings occurred at high levels of Al, Cu and Pb.

We chose image analysis to quantify the impact of the metal exposures on wild rice. This technique enabled non-destructive measurement of the seedlings at periodic intervals throughout the experiments. The method was particularly suitable for wild rice because its roots tend to spiral when exposed to light. A simple root length measurement would therefore not be appropriate. The method also permits a visual record to be kept of the plants throughout the experiment.

There was a great deal of "raw data" generated in the preparation of the report. This includes chain of custody forms for all chemical analyses (we have included a sample copy in the Appendix), measurements of the 4500 seeds tested for size suitability, and image analysis data for all plants measured throughout the experiments. We also have the 900 images of the exposed samples in digital format. This information will be kept on file at the university. Should you require any of this material, contact me at any time.

In closing, I would like to say that we found the project exciting and the results somewhat startling. Although I realize the purpose of the study was for a specific problem, the implications of the findings are considerable. Clearly plants in general, and wild rice specifically, should be considered in any industrial discharge scenario. Hopefully future studies will further examine these important considerations.

Sincerely yours,

Peter Lee

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Dr. P.F. Lee
July 20, 1998

Ann McCammon Soltis
Policy Analyst
Great Lakes Indian Fish and Wildlife Commission
P.O. Box 9
Odanah, Wisconsin 54861
USA

Dear Ann:

As we discussed previously, the concentration of Al where adverse effects on wild rice were first detected was 1.0 ppm, not 10.0 ppm as erroneously stated in the summary, on page 47 of the discussion and in the conclusions and recommendations of the report entitled "The Effect of Heavy Metals on the Early Growth of Wild Rice". Similarly the correct value where Al was toxic to duckweed was 1.0 not 10.0. Table 7 of the same report details the correct results for Al wherein Treatment 4 (1.0 ppm) had statistically significant lower ($P < 0.5$) leaf and root areas from Treatment 1 (control - 0.0 ppm Al), Treatment 2 (0.01 ppm Al) and Treatment 3 (0.1 ppm Al). The correct value of 1.0 ppm is also stated in the published account of this protocol listed below:

Lee, P.F. and P.C. Hughes. 1998. A plant bioassay protocol for sediment heavy metal toxicity studies using wild rice as an indicator species. Proc. 2nd Biennial International Conference on Chemical Measurement and Monitoring of the Environment. Ottawa, Canada.

I apologize for any confusion these errors may have caused. If you require further clarification on the findings of this report, I would, of course, be pleased to assist you in any way I could.

Sincerely yours,

Peter Lee

INTRODUCTION

STATEMENT OF THE PROBLEM

Wild rice has been an important part of the history and culture of the Chippewa Indians of Wisconsin for centuries (Beck, 1994). One of the major wild rice stands harvested by the Chippewa is located at Mole Lake near Crandon, Wisconsin and is controlled by the Mole Lake Band.

Exxon and Rio Algon Ltd. propose to construct a mine in the Swamp Creek - Wolf River Watershed adjacent to the Mole Lake Indian Reservation and immediately upstream of the Mole Lake wild rice stand. As a result of the mine construction, it is feared that there may be changes to the hydrology and water quality that could detrimentally affect wild rice growth on Mole Lake. The most likely changes to water quality include elevated levels of sulphur and heavy metals.

NUTRIENT RELATIONSHIPS OF WILD RICE

General descriptions of wild rice and its characteristic habitat have been published by Fyles (1920), Chambliss (1940), Moyle (1944), Steeves (1952), Dore (1969), and Aiken et al, (1988).

Moyle's (1944) study on the distribution of aquatic plants in Minnesota was the first to deal with the nutrient relationships of wild rice. Moyle observed that wild rice tolerated alkalinity ranges of 5 - 250 ppm but that no large wild rice stands were found in waters with sulphate concentrations greater than 10 ppm and rice was generally absent from waters with sulphate levels greater than 50 ppm. Other studies which have included observational data (Pip and Paulishyn, 1971; Lee and Stewart, 1981) and experimental data (Vicario and Halstead, 1968; Lee, 1979) have suggested that sulphate in concentrations up to 250 ppm had little effect.

Studies on the ecology of other elements versus wild rice have been less defined. Lee and Stewart (1983) modeled the seasonal uptake of nutrients in a eutrophic river system. This study showed that most nutrients were taken up by wild rice at a constant rate per unit weight. The notable exception was for metals which were present in luxury concentrations. Other investigations have examined the nutrient requirements of wild rice and its growth in different types of sediment.

Within-population variations in wild rice could be correlated to relatively uniform nutrient regimes, "environmental regions", that could be identified in individual lakes using Q-type cluster analysis (Lee, 1986; Keenan and Lee, 1988). Among-population variations in wild rice production were also related to sediment type which were classified as either organic, flocculent or clay (Day and Lee, 1989). Production was poorest in flocculent sediments and detailed studies of this soil type revealed that nutrient deficiencies occurred in wild rice seedlings after seed reserves were depleted, and controlled experiments revealed that mineral deficiency symptoms were visually evident at later stages in its life cycle (Day and Lee, 1990).

There have been no published studies conducted on the effects of toxic levels of nutrients on wild rice growth. Unpublished work by Lee while examining the impact of various types and levels of fertilizers on wild rice showed that high levels of metals could cause chlorosis and necrosis of wild rice seedlings that eventually led to death.

Complicating all investigations on the effects of nutrients on wild rice have been the high levels of genetic variations in such a wild species. Counts and Lee (1988, 1990) showed that among population variations in flowering time, biomass, height, leaf width and floret production were genetically influenced. Within population variation was similarly high and variations in seed size were shown to influence the early development of wild rice (Counts and Lee, 1991).

DEVELOPMENT OF TOXIC ASSESSMENT METHODOLOGY FOR WILD RICE

The discussion above indicated that there is considerable information on the nutrient ecology of wild rice, but very little dealing specifically with toxicity effects and a methodology must be devised to quantify any toxic effects. Pertinent information for this purpose can be derived from the following three sources:

1. Toxic Effects in Other Wild Species

Studies on populations of wild species including Festuca ovina (Snaydon and Bradshaw, 1961), Trifolium repens (Snaydon and Bradshaw, 1969), Anthoxanthum odoratum (Davies and Snaydon, 1974), and Chionochloa (Chapin et al, 1982) have demonstrated that these species can survive under nutrient stressed conditions. Typically the stressed plants exhibited low growth rates and high elemental concentrations per unit biomass.

2. Screening for Stress Tolerant Cultivars

Of considerable application in assessing the effects of toxic elemental concentrations on wild rice are the techniques devised to screen cultivars of various crops for their tolerance to nutrient stressed conditions. Devine (1982) reviewed the screening methodologies commonly used for selecting nutrient tolerant plants. The majority of the techniques are based on cultivating plants in nutrient stressed soil or solution culture, and selecting superior varieties based on their dry weights (or some other measure of plant performance including spectral reflectivities), root-shoot ratio, root area, or their nutrient efficiency. According to Devine, a reliable assay should simulate the appropriate stress, characterize the genotype of the zygote without any maternal influences from seed reserves, and be rapid and inexpensive. Typically screening for nutrient tolerance is done at the seedling stage after a correlation has been established between the indicator response at earlier phenological stages, and the performance of mature individuals. Howeler and Cadavid (1976) provide a good example of this approach. After establishing a correlation between root length of rice seedlings and performance in the field, they were able to identify an aluminum-tolerant line from 200 cultivars. Assessment of large numbers of seedlings can be facilitated with computerized image analyses (Ottman and Timm, 1984).

3. Bioassays for Allelopathy and Toxic Effects Using Aquatic Plants

Bioassays are commonly used to test for allelopathy in aquatic plants and to examine the effect of toxicants on aquatic plants.

Allelopathy bioassays are generally conducted by exposing either lettuce seeds or Lemna spp. to organic compounds that have been extracted from suspected allelopathic aquatics. The bioassay for lettuce seeds is the percent germination and/or the length of the root radicle in comparison to control treatments. The bioassay for Lemna spp. is the growth of new fronds relative to the control. Of the two, the lettuce seed bioassay has generally been accepted as the best test subject (Elakovitch et al, 1989; Sutton and Porter, 1989).

Aquatic macrophytes have been proposed as good substitutes for common fish and invertebrate LC50 assessments because of their essential position in aquatic ecosystems and their ease of use in bioassays. The most common macrophytes used for this purpose are Lemna gibba and Lemna minor (Wang, 1990). A standard protocol for using Lemna spp. has been developed by the American Public Health Association. Other species that have been tried include cucumber, lettuce and millet (Wang, 1987). It is noteworthy that wild rice is among the other species recommended for testing this procedure (Wang, 1990). The bioassay for these species include root elongation, frond growth or chlorophyll production (Wang, 1986, 1987; Taraldsen and Norberg-King, 1990).

Perhaps the most important aspect of these bioassays, relative to the present proposed study, is the evidence that suggests that chronic values derived from aquatic animal toxicity tests are not always sensitive to protect aquatic plants in the receiving water (Taraldsen and Norberg-King, 1990). Wang (1990) compared fish versus duckweed bioassays on metal toxicities and found duckweed to be more sensitive than fish for Cd, Cr, Pb, Ni, and Se, and comparable for Cu and Zn.

SIGNIFICANCE OF LITERATURE REVIEW TO PRESENT STUDY

The following pertinent factors concerning the proposed study can be extracted from the above literature review:

1. Wild rice exhibits luxury consumption of metals and therefore changes in the concentrations of these metals would be expected to impact on wild rice.

2. Visual nutrient deficiency symptoms occur in wild rice after the seed reserves have been exhausted. No research has been conducted on effects of nutrient imbalances before this has occurred.

3. Screening procedures for selecting stress tolerant cultivars in other grains are usually conducted at early phenological stages, and the same methodology should be appropriate for wild rice.

4. Bioassays for heavy metals have been successfully conducted using aquatic macrophytes. Wild rice has been mentioned in the literature as a possible candidate for this technique.

5. A standard protocol exists for bioassays using Lemna spp., and this protocol could be modified for wild rice.

OBJECTIVES

This research examined whether the early development of wild rice was affected by elevated heavy metal concentrations. Specifically, the objectives were (a) to develop a bioassay for wild rice modeled after methods used for other aquatic macrophytes; and (b) to use this bioasssay to determine the threshold levels of Al, Cd, Cu, Pb, and Hg that affect the early growth of wild rice.

METHODS

A. WILD RICE

A randomized block experimental design was used for each element, with two series of experiments containing three replicates of six treatments. The bioassay measured the growth performance of the wild rice seedlings. At the completion of the experiments, chemical and data analyses assessed any chemical and/or biological differences of the wild rice seedlings among the various treatments.

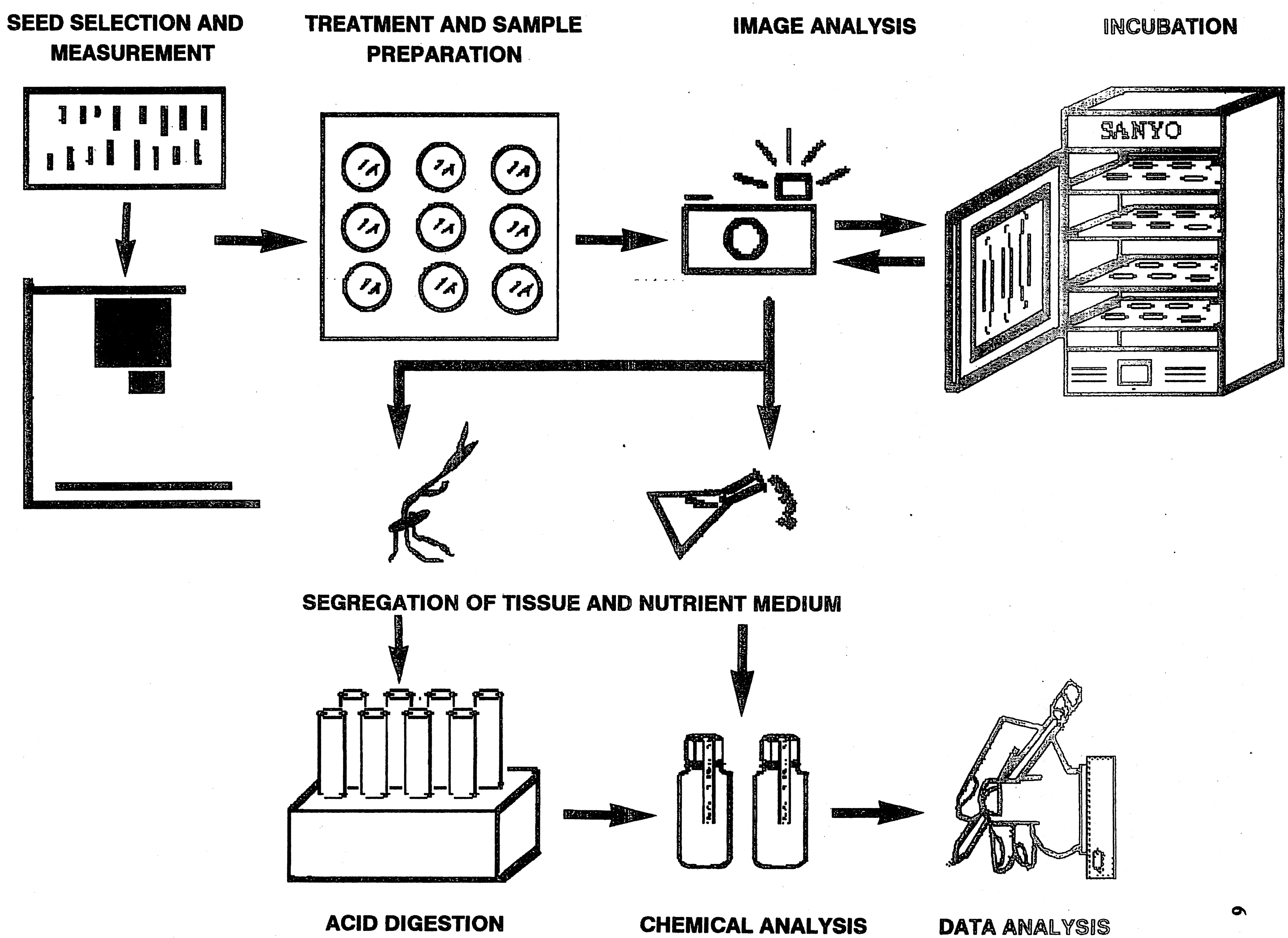
Image analysis was used to measure growth performance of the wild rice seedlings. This technique enabled non-destructive measurements to be taken throughout the course of the experiments and permitted more accurate quantification of individual plant differences than simple destructive weighing.

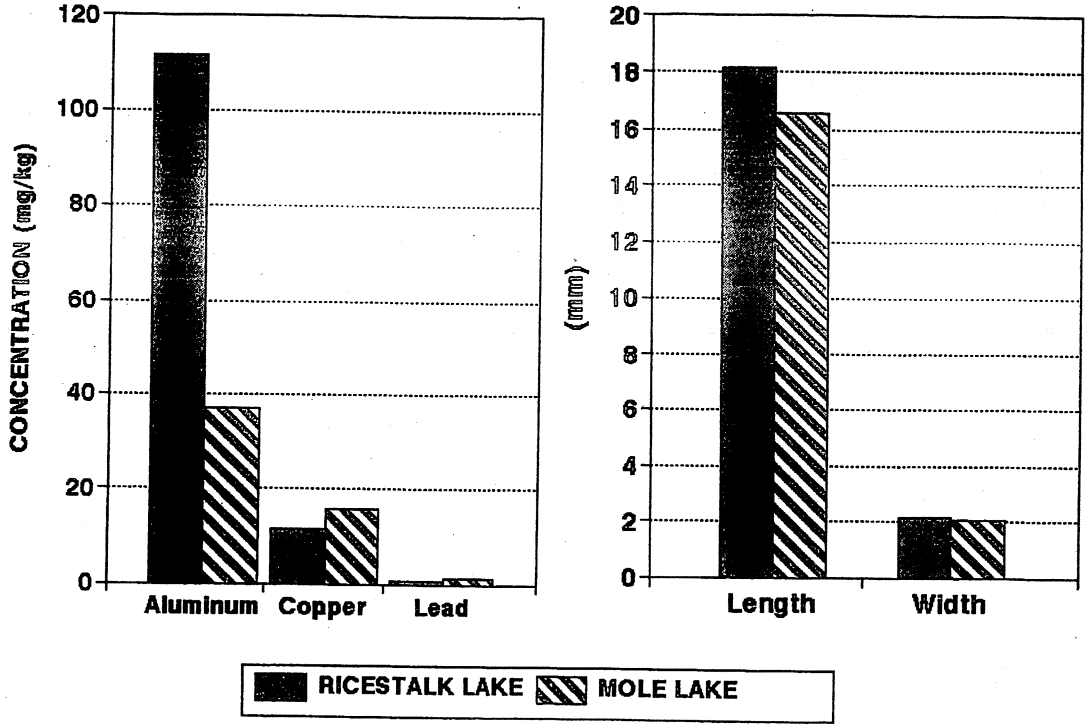
BIOASSAY

The basic procedure shown overleaf, was developed for the bioassay. Each of the main steps are detailed below.

1. Seed Selection and Measurement

Viable wild rice seed from Mole Lake was not available for the bioassays and it was necessary to use seed collected from Ricestalk Lake in Northwestern Ontario (49° 6' 90° 9'). Seed characteristics of Ricestalk vs. Mole Lake are shown in Chart 1. Ricestalk Lake differed significantly from Mole Lake in length, width, and Al content. Since seed size is known to affect the early development of wild rice (Counts and Lee, 1991), selection of seed of the same size was desired.


SEED SELECTION AND MEASUREMENT
TREATMENT AND SAMPLE PREPARATION
IMAGE ANALYSIS
INCUBATION
SANYO
SEGREGATION OF TISSUE AND NUTRIENT MEDIUM
ACID DIGESTION
CHEMICAL ANALYSIS
DATA ANALYSIS



Seed Source	Sample	Aluminum	Copper	Lead	Length	Width
			(mg/kg)		(mm)	
Ricestalk	***1***	58.46	10.42	< 0.02		
	2	69.66	10.21	< 0.02		
	3	167.86	10.86	1.04		
	4	150.37	14.37	1.33		
	Mean	111.59	11.47	0.59	18.14	2.15
Mole Lake	***1***	23.82	12.36	1.86		
	2	60.44	22.50	1.25		
	3	41.06	15.18	2.00		
	4	22.87	12.61	< 0.02		
	Mean	37.25	15.66	1.28	16.55	2.08

Chart 1: Ricestalk vs Mole Lake: a comparison of seed length, width and initial concentrations of test metals.

In this case, the seed size used was not to exceed +/- 10% of the mean value of length and width for Mole Lake seed.

The seed was measured with the wild rice grading system at the CLWR. This equipment was developed to grade Canadian Lake wild rice based on length, width and colour. Essentially the apparatus consists of a seed stage which moves by computer under a video camera which is contained within a lighted aluminum enclosure. This enclosure ensures uniform light conditions. The seed stage moves exactly one frame at a time. At each frame stop, 8 seeds are simultaneously photographed by the camera and the image is sent to a frame grabber on board a personal computer. Custom software analyzes each seed for its size and colour and outputs the results to a printer or file. Using this procedure, some 4500 seeds were measured in order to meet the size requirements for sufficient seed for the experiments.

2. <u>Treatment and Sample Preparation</u>

Six treatments were prepared for each element to cover a range of concentrations from 0 to levels that were estimated from the literature to be toxic to plants. As suggested in Standard Methods of the American Public Health Association (18th edition) for examination of aquatic plants, concentrations were increased logarithmically (base 10) between treatments. The treatment concentrations for each element are shown below:

Treatment Concentrations Used in Experimentation

	Treatment Concentration in ppm					
	1	2	3	4	5	6
ALUMINUM	0	0.01	0.1	1.0	10.0	100.0
COPPER	0	0.001	0.01	0.1	1.0	10.0
CADMIUM	0	0.0001	0.001	0.01	0.1	1.0
LEAD	0	0.001	0.01	0.1	1.0	10.0
MERCURY	0	0.0001	0.001	0.01	0.1	1

The wild rice growth medium used was that developed by Malvick and Percich (1993). Volumes of the metal solutions were added to this solution to achieve the above concentrations. The exception to this was for Cu which is an essential growth element and was already present in the wild rice growth medium. In this case, volumes of copper solution were added to increase the nutrient solution by the above amounts for copper.

3. Incubation of Samples

Seeds were germinated in distilled, deionized water at 16°C under conditions of 12 hours of light and 12 hours of darkness. Light intensity was set at approximately 125 $uE/m^2/s$. A sample consisted of 50 ml of solution (nutrient + metal addition) in a 100 mm diameter petri plate into which were placed two wild rice seedlings that were one day after germination in age. Samples for each treatment were placed on 45 cm x 45 cm sheets and these were set on shelves inside a Sanyo Model MLR350 plant growth chamber. Germination settings were maintained for the test.

4. Scanning and Image Analyses of Samples

All samples were scanned initially and at three day intervals using a Howtek 3+ flatbed scanner at an intensity of 3480 pixels per cm^2. Image analysis of the scanned images then determined the surface area of leaves and roots as well as the reflectance brightness values of red, green and blue light. Area, rather than length of root, was selected since wild rice roots tend to spiral when exposed to light and thus a length measurement has little meaning.

5. Chemical and Data Analysis

At the completion of the experiments, both treatment solutions and wild rice seedlings were tested for their metal content. With the exception of Hg, all solutions and plant tissues were analyzed at the Lakehead University Environmental Laboratory. Mercury in solution was measured by Accurassay Laboratories and mercury in plant tissue was measured by Envirotech Laboratories, both located in Thunder Bay, Ontario. All laboratories followed the protocols of the Ontario Ministry of Environment and Energy. Chain of custody forms traced the transfer of all samples.

For the first three treatment solutions of Cd, Pb, Cu, and Al, each sample was concentrated to 10 ml in 2.5 cm x 20 cm glass tubes in a Skalar aluminum digesting block, Model 5620/40, at 130°C prior to analysis. Since the weight changes in the individual plants were not much greater than the accuracy of the Mettler Model AC100 used for weighing (+/- 10 mg), the three replicate plant samples for each treatment were combined and then ashed overnight at 550°C. The ashed samples were dissolved in 1 ml of concentrated HNO_3 and 3 ml of concentrated HCl and then brought up to 10 ml with distilled, deionized water. Analyses of all elements were performed on a Jerrell-Ash 9000 ICP. Five quality control standards and 4 blanks were measured with every set of treatment analyses for each element.

Data analysis proceeded in four steps:

1. since each image analysis sample consisted of two plants with differing areas of leaves and roots, brightness values for red, green and blue were corrected to represent values per unit area.

2. in order to better assess the treatment effects, variation among individual samples within each treatment were reduced by calculating the change in leaf and root areas between sample times. This change in area rather than the actual area at any one point in time was thought to be more representative of the treatment effect and reduced the natural variation that is present within such a wild population.

3. since the growth rate of wild rice is not constant during its early development, comparisons among treatments between times are best done by using a time-independent transformation (Lee and Stewart, 1981). Essentially this transformation changes such data which is additive in a statistical sense, to a relative rather than an absolute form. That is, what is important is not the actual value, but the value of one sample relative to that of another.

4. anova's of the time corrected area data, and the red, green, blue brightness data for each element were calculated for each series x treatment x replicate. In the cases where the series were not significantly different, least significant difference (LSD) comparison of means determined which treatment results within each elemental experiment were different from each other.

B. LEMNA

The effects of the treatment solutions on Lemna were tested using the wild rice experimental design, with six treatments x 3 replicates for each element. These experiments were not repeated in a second series as they were with wild rice.

The effects of the treatments on Lemna were measured by counting the number of fronds, the number of broken colonies, and the presence or absence of chlorosis as outlined by Wang and Williams (1990).

RESULTS

A. WILD RICE

The effects of test metals on wild rice are summarized in the following pages. Levels of toxicants found in the seedlings and nutrient solutions on Day 13, are recorded in Tables 1-5. Scanned images of typical test seedlings for each metal and treatment have been reproduced in colour Plates 1-5. Figures 1, 4, 7, 10 and 13 illustrate changes in leaf and root area. Changes in leaf red, blue and green spectral reflectivities are illustrated in Figures 2, 5, 8, 11 and 14. Spectral changes for roots are described by Figures 3, 6, 9, 12 and 15. Table 7 documents statistically significant differences in area and colour between treatments. Tables, Plates and Charts are arranged by toxicant to facilitate easy access.

ALUMINUM

Growth performance of wild rice in the six aluminum treatments is shown in Plate 1 and Figures 1 to 3.

Plate 1 visually illustrates the differences that existed among the six treatments. The absence of shoot and root growth is evident in Treatments 5 and 6. Plants were dead in Treatment 6. Shoot size is much smaller in Treatment 4 where root production is also greatly reduced from Treatments 1 to 3.

Areas of leaves and roots (Fig. 1) tended to be lower in Series 1 than Series 2 possibly because of the location of the first series near the bottom of the growth chamber where light levels seemed to be slightly less intense. However the same patterns existed in both series. In both leaf and root areas, Treatments 1 , 2, and 3 had higher values than Treatment 4. This growth difference was accentuated for Treatment 5 and Treatment 6 where no roots were present and the seedlings were dead. These patterns for area differences were evident by the fourth sampling period (Day 10 from the start of the experiments).

There was a tendency for red, green and blue brightness of leaves to increase in Treatments 5 and 6 (Fig. 2). These higher values were due to chlorosis of the leaves and were noticeable by the second sampling (Day 4 from germination) period. Although less evident, brightness values for Treatment 6 in the roots (Fig. 3) were also higher, again due to the loss of colour.

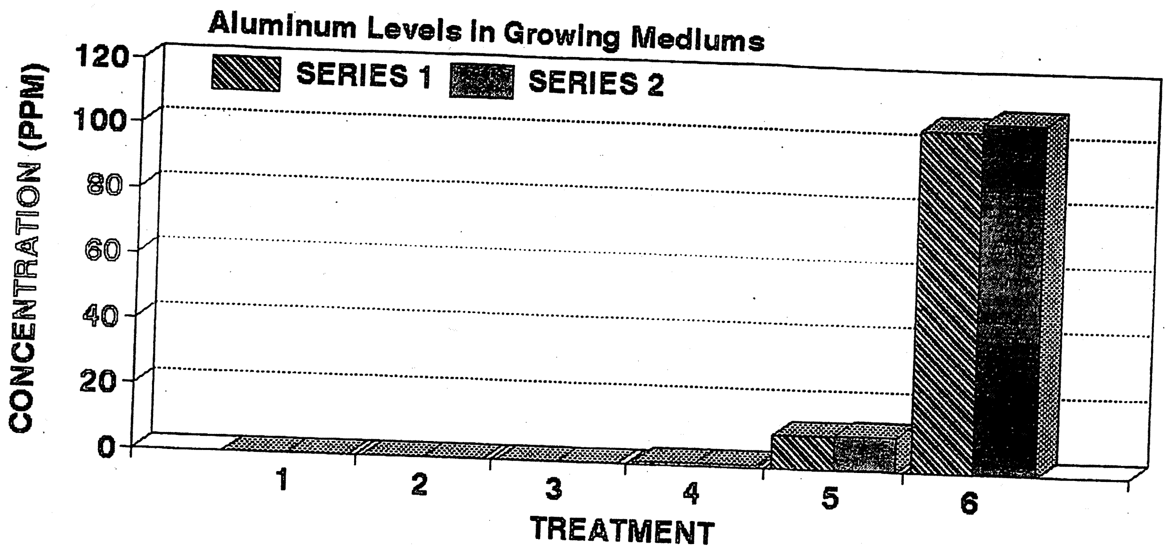
Paired comparison tests of leaf and root areas and leaf and root brightness values between treatments are shown in Table 7. Leaf areas were significantly different between Treatments 1, 2, 3 and Treatments 4, 5, and 6. Treatments 5 and 6 were not significantly different between each other. These same differences among groups were present for root area. Red and Green brightness values differed among experimental series, and therefore only differences between Blue brightness treatment values were calculated which followed the same pattern as did leaf and root area. Root brightness values were different for Treatment 6 for red, blue and green. The other treatments tended to have combinations of differences which followed no definite pattern.

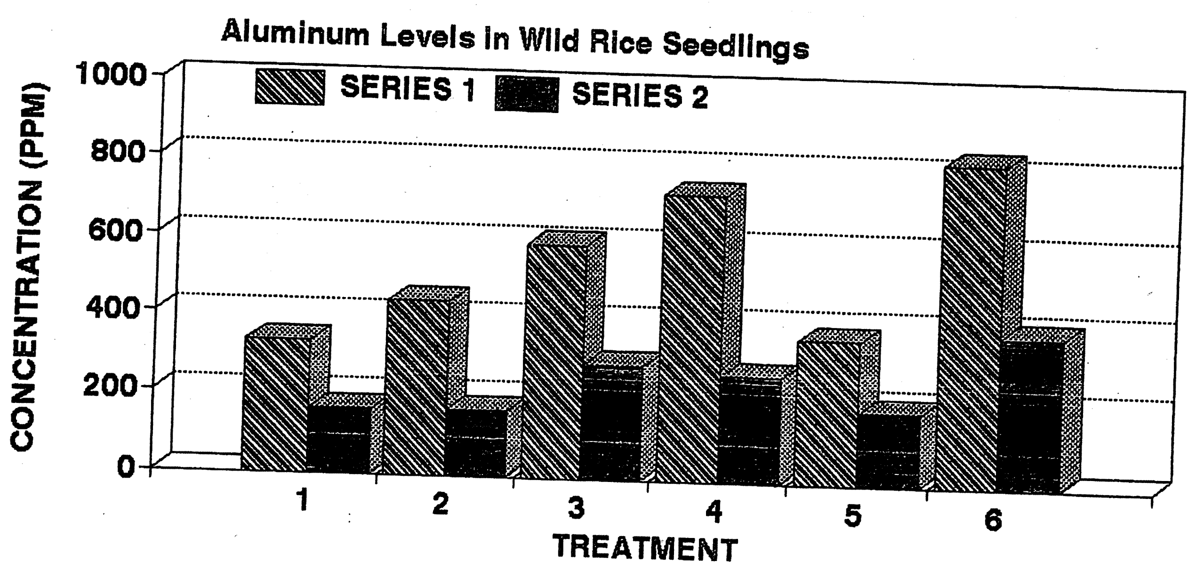
Levels in Growing Medium and Seedlings

The Al levels in the growing medium and the plant seedlings at the end of the growing season are shown in Table 1. Al levels varied little between the two growing mediums, but were much higher in plant tissue in Series 1 vs. Series 2. This was likely due to the smaller size of the plants in the first series noted above which were phenologically retarded relative to Series 2. Previous research (Lee and Stewart, 1983), showed that levels of most elements decrease as the rice plants develop. Both series did have the same trend of increasing in concentration in the tissue as the concentration of the solution increased.

SUMMARY

Under controlled conditions in a growth chamber, wild rice seedlings were exposed for 13 days to concentrations of Al, Cd, Cu, Pb, and Hg added to wild rice nutrient solution. At three day intervals, the samples were removed and image analysis was used to quantify leaf and root area, and red, blue and green colour reflectance. At the end of the experiments, the plant tissue and nutrient solution were analyzed for the element of interest. Data analysis determined which concentrations of each element significantly affected wild rice growth performance. The chemical analyses showed that the contaminants were present in the solution, and wild rice took up these contaminants into its tissue. The most noticeable effects for all elements were the reduction of leaf and root growth at higher treatment levels. In the case of Al, Cu, and Pb, mortality occurred at the highest treatment level. Chlorosis of leaves and roots also occurred. These patterns were evident after seven days of exposure. Concentrations (ppm) at which adverse effects were initiated in wild rice were: Al, 1.0; Cu, 1.0; Cd, 0.01 (leaves), 1.0 (roots); Pb, 1.0; and Hg, 1.0. Common duckweed was also grown under the same contaminant levels as wild rice. The levels of contaminants affecting this species were similar to wild rice with the exception of Pb to which wild rice was much more sensitive.





TREATMENT	SERIES	WATER ANALYSIS		TISSUE ANALYSIS
		MEAN	STD	MEAN
1	1	< 0.05	n/a	331.18
	2	< 0.05	n/a	158.73
2	1	< 0.05	n/a	440.14
	2	< 0.05	n/a	161.14
3	1	< 0.05	n/a	590.20
	2	< 0.05	n/a	284.62
4	1	0.637	0.027	723.90
	2	0.450	0.005	263.51
5	1	10.223	0.488	361.14
	2	10.500	0.191	176.39
6	1	105.544	1.528	818.78
	2	108.150	2.780	378.59

Table 1: Aluminum detected in wild rice seedlings and growing mediums in ppm.

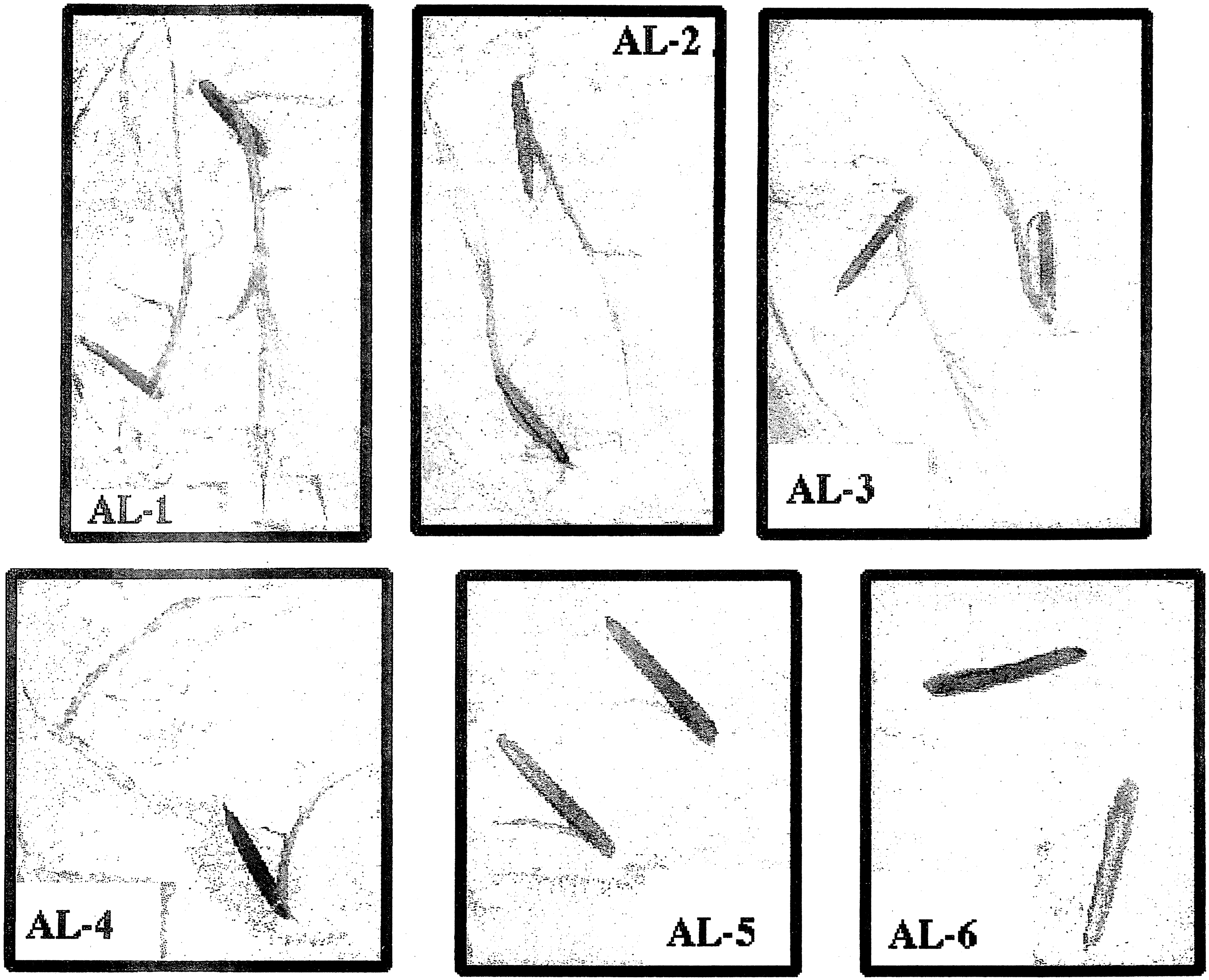


PLATE 1: Typical appearance of wild rice seedlings exposed to Al treatments for 13 days.

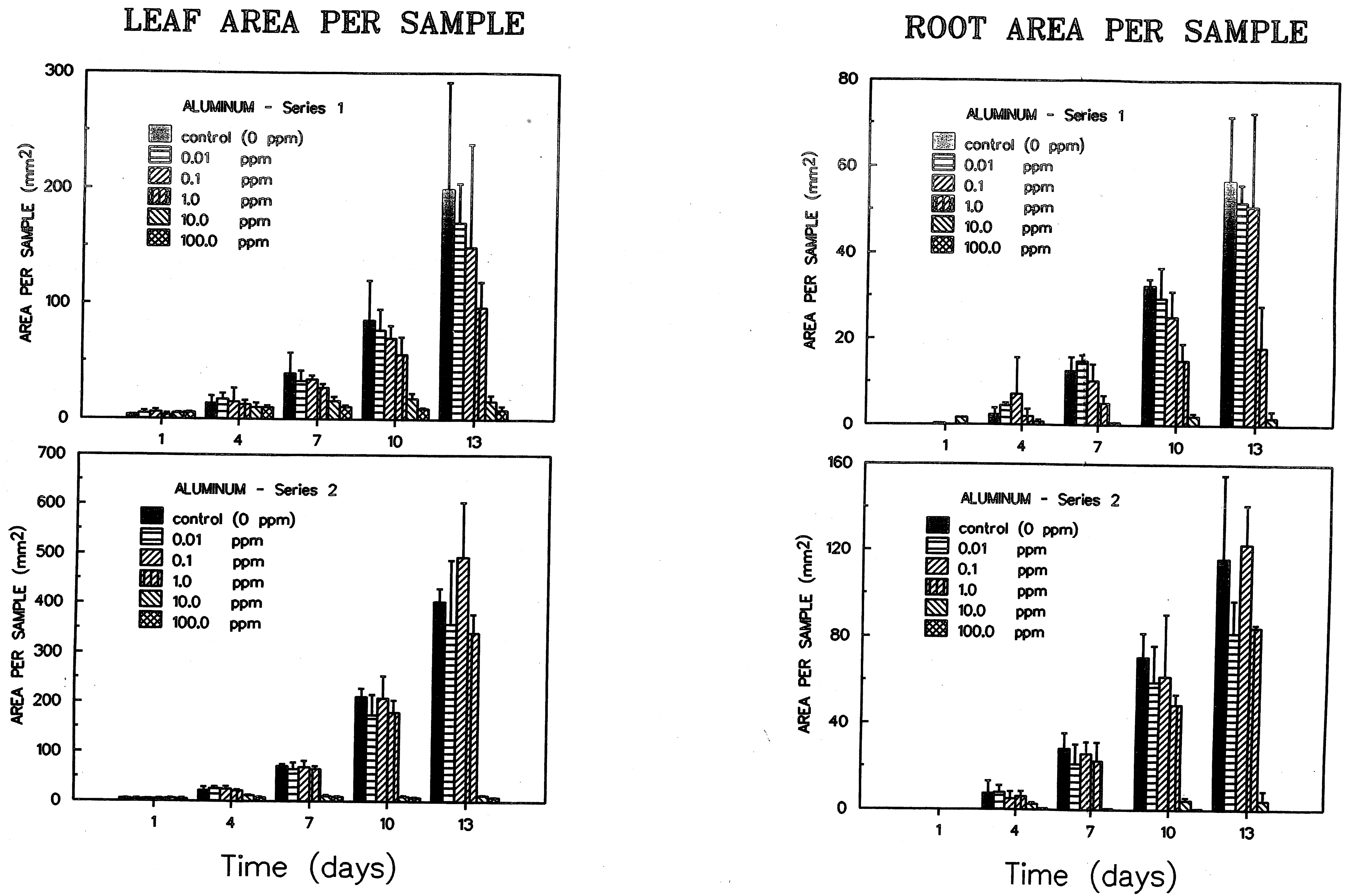


Figure 1: Leaf and root area of wild rice seedlings exposed to Aluminum treatments.

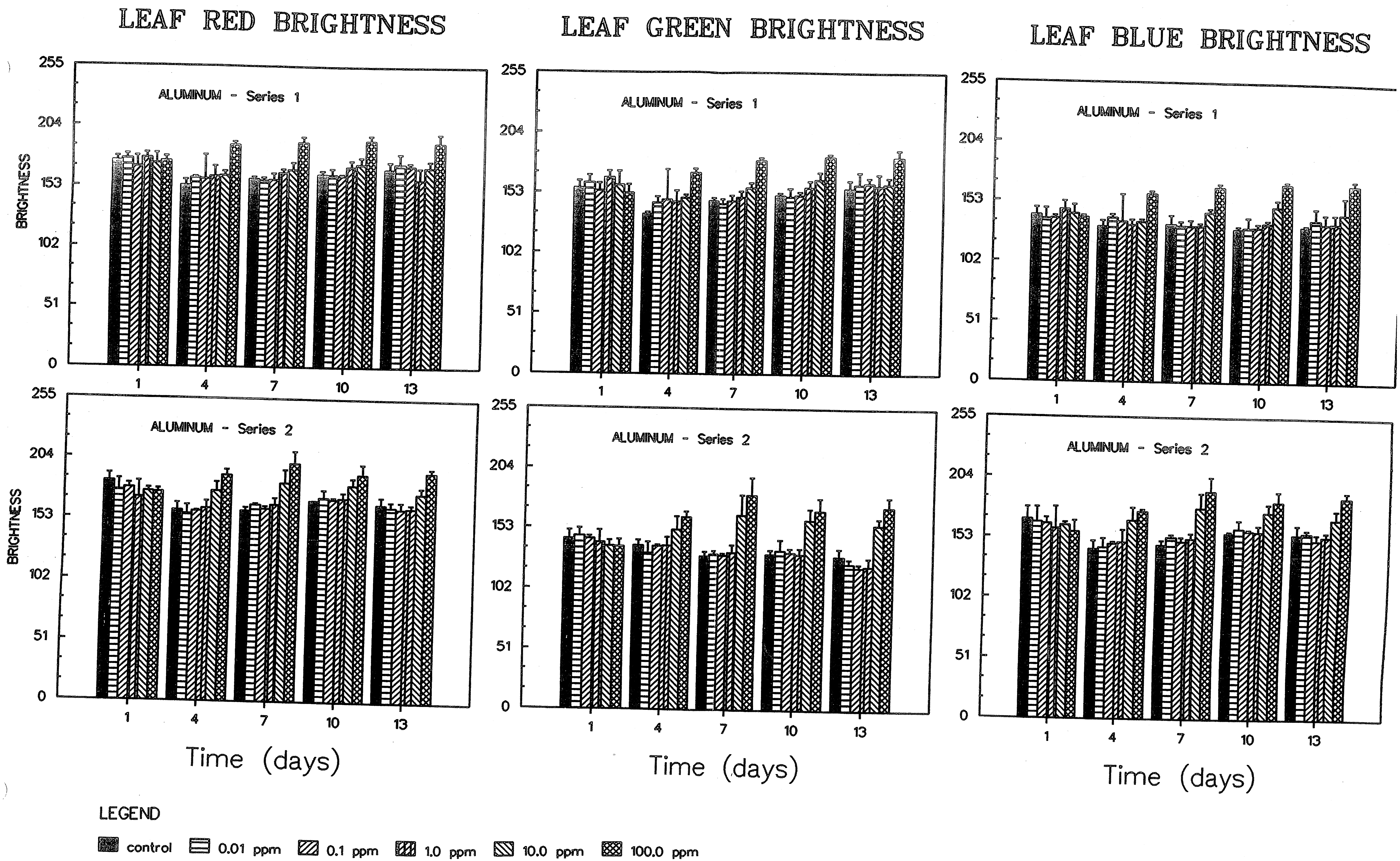


Figure 2: Leaf red, green and blue spectral values for wild rice seedlings exposed to Aluminum treatment

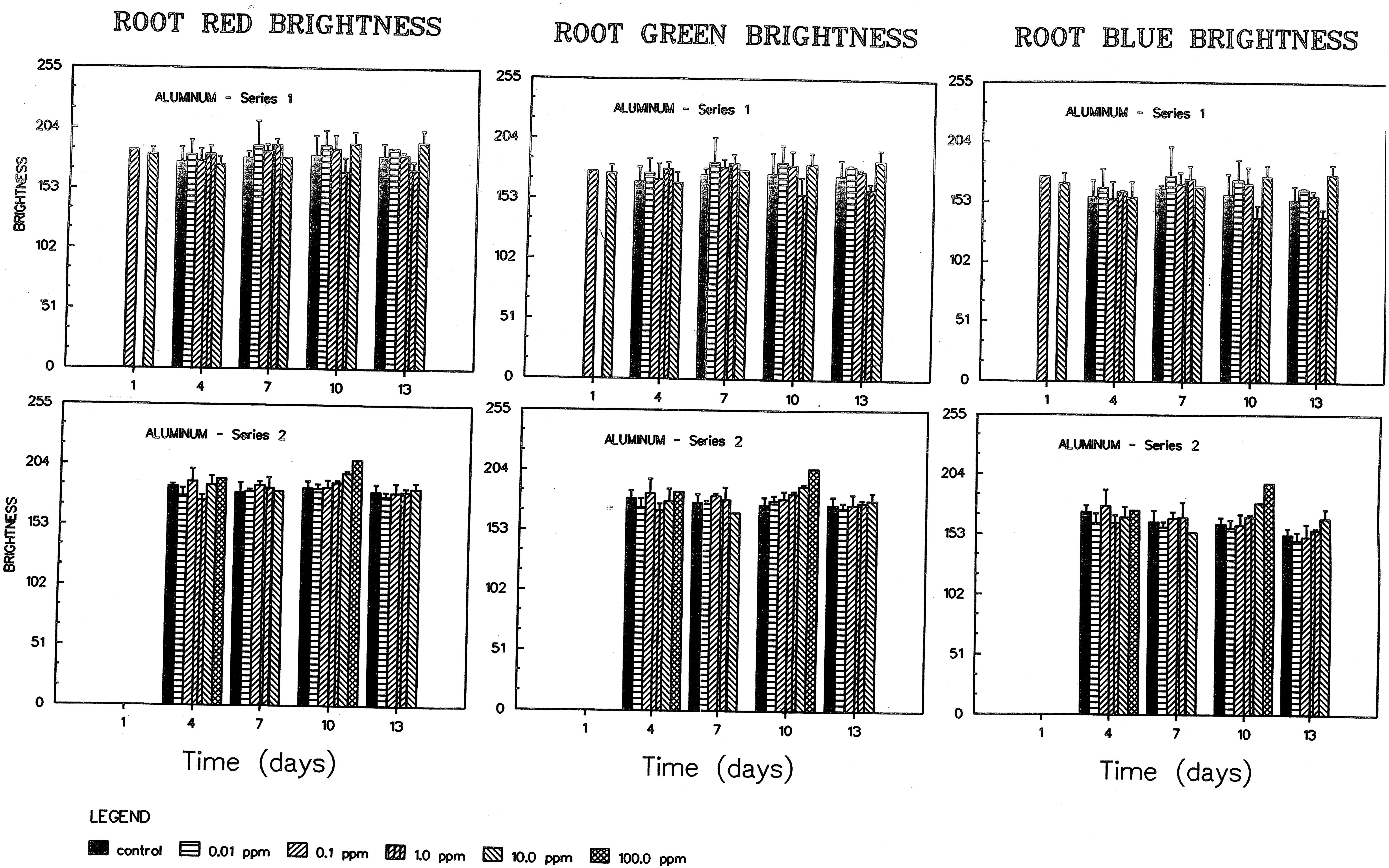


Figure 3: Root red, green and blue spectral values for wild rice seedlings exposed to Aluminum treatments.

COPPER

Growth performance of wild rice in the six copper treatments is shown in Plate 2 and Figures 4 to 6.

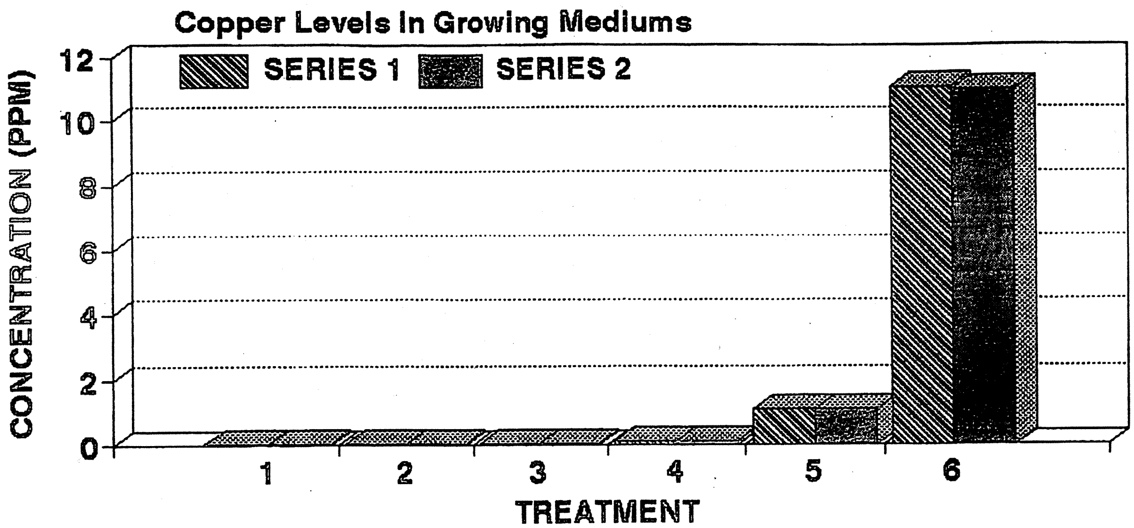
Plate 2 visually shows the differences among the six treatments which were evident by the third sampling period. Most noticeable is the absence of shoot or root growth in Treatment 6 where mortality occurred. Although shoot growth occurred in Treatment 5, root growth was reduced or absent.

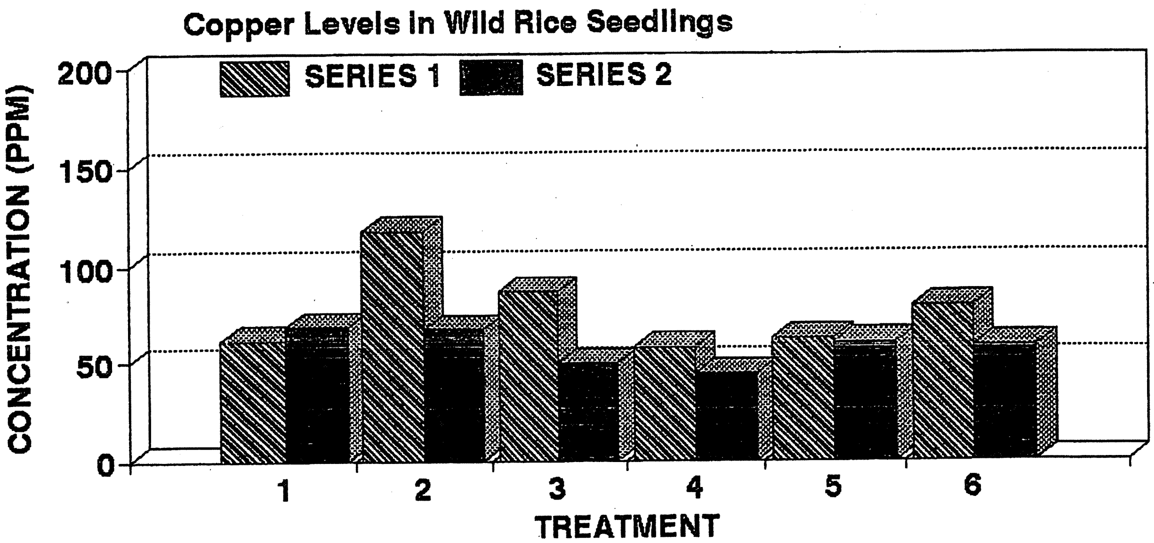
Areas of leaves and roots (Fig. 4) were similar for Treatments 1 to 4, and were much higher than Treatments 5 and 6. Treatment 5 had higher values for leaf area than Treatment 6, but similar root area values. This pattern was evident by the third sampling period (Day 7 from the start of the experiments).

Paired comparison tests of leaf and root areas and leaf and root brightness values are shown in Table 7. Leaf areas were significantly different between Treatment 6 (where the plants died) and all other treatments. Of the surviving treatments, Treatment 5 had significantly lower leaf areas than Treatments 1 to 4. Root production was impeded in Treatment 5 and root area values were therefore the same as Treatment 6. Chlorosis was evident in the shoots and roots of Treatment 6, causing it to have significantly different values for red and green brightness values than the other treatments. In terms of root brightness values, root production that did occur in Treatment 5 was chlorotic and the colour brightness values were therefore statistically different from the other treatments.

Levels in Growing Medium and Seedlings

Similar levels of Cu were found in the growing mediums and seedlings of Series 1 and 2 (Table 2). There was no pattern of increasing concentration of copper in the seedlings vs. copper added in solution. This was likely a result of the concentrations of copper naturally present in the seed and the copper added as part of the growing medium.





TREATMENT	SERIES	WATER ANALYSIS		TISSUE ANALYSIS
		MEAN	STD	MEAN
1	1	0.012	0.003	61.56
	2	0.012	0.002	68.40
2	1	0.011	0.001	117.56
	2	0.011	0.003	66.73
3	1	0.019	0.003	86.44
	2	0.018	0.002	49.59
4	1	0.099	0.006	57.60
	2	0.104	0.009	43.44
5	1	1.101	0.032	62.23
	2	1.098	0.040	59.96
6	1	11.120	0.185	78.65
	2	11.008	0.194	57.89

Table 2: Copper detected in wild rice seedlings and growing mediums in ppm.

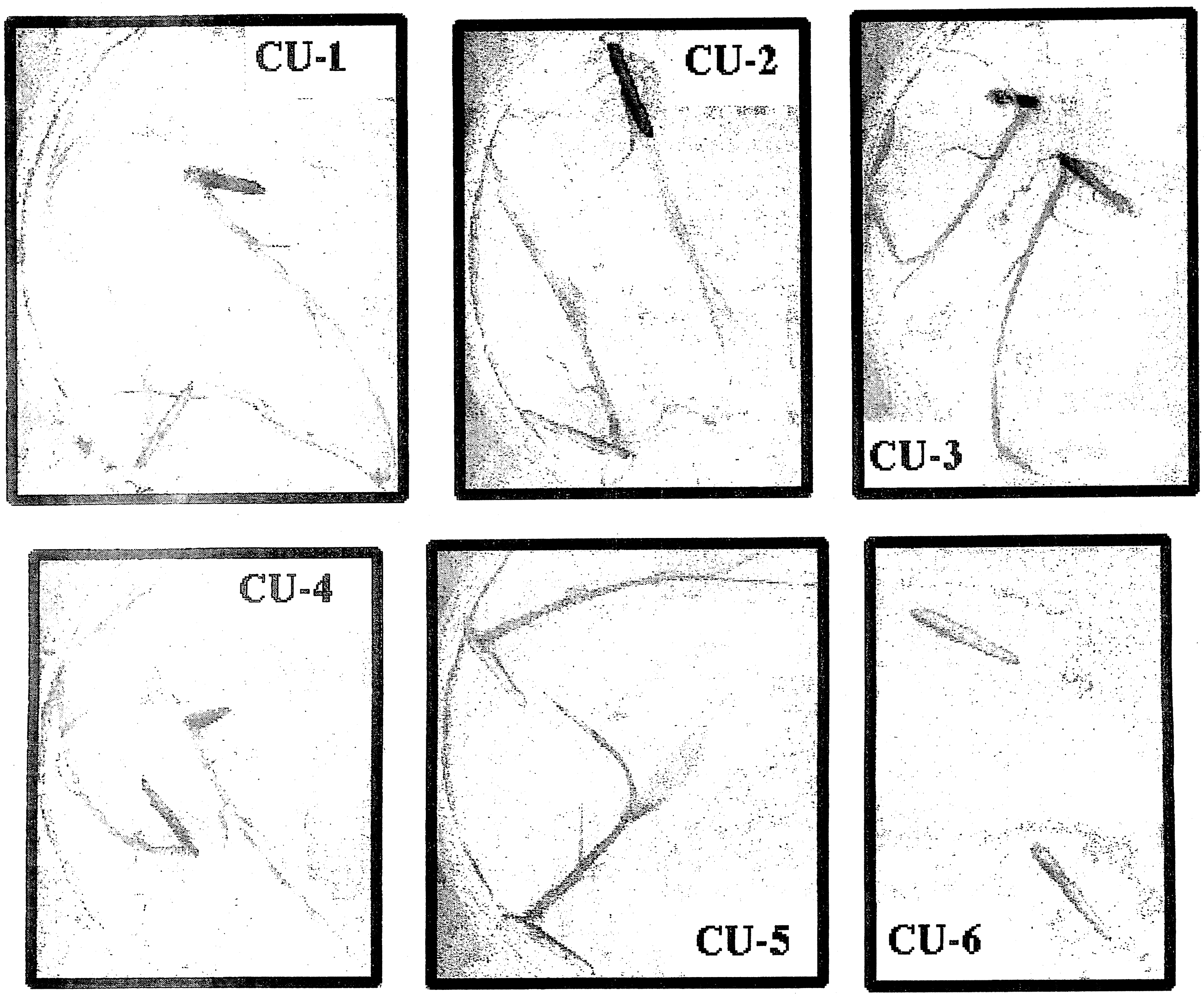


PLATE 2: Typical appearance of wild rice seedlings exposed to Cu treatments for 13 days.

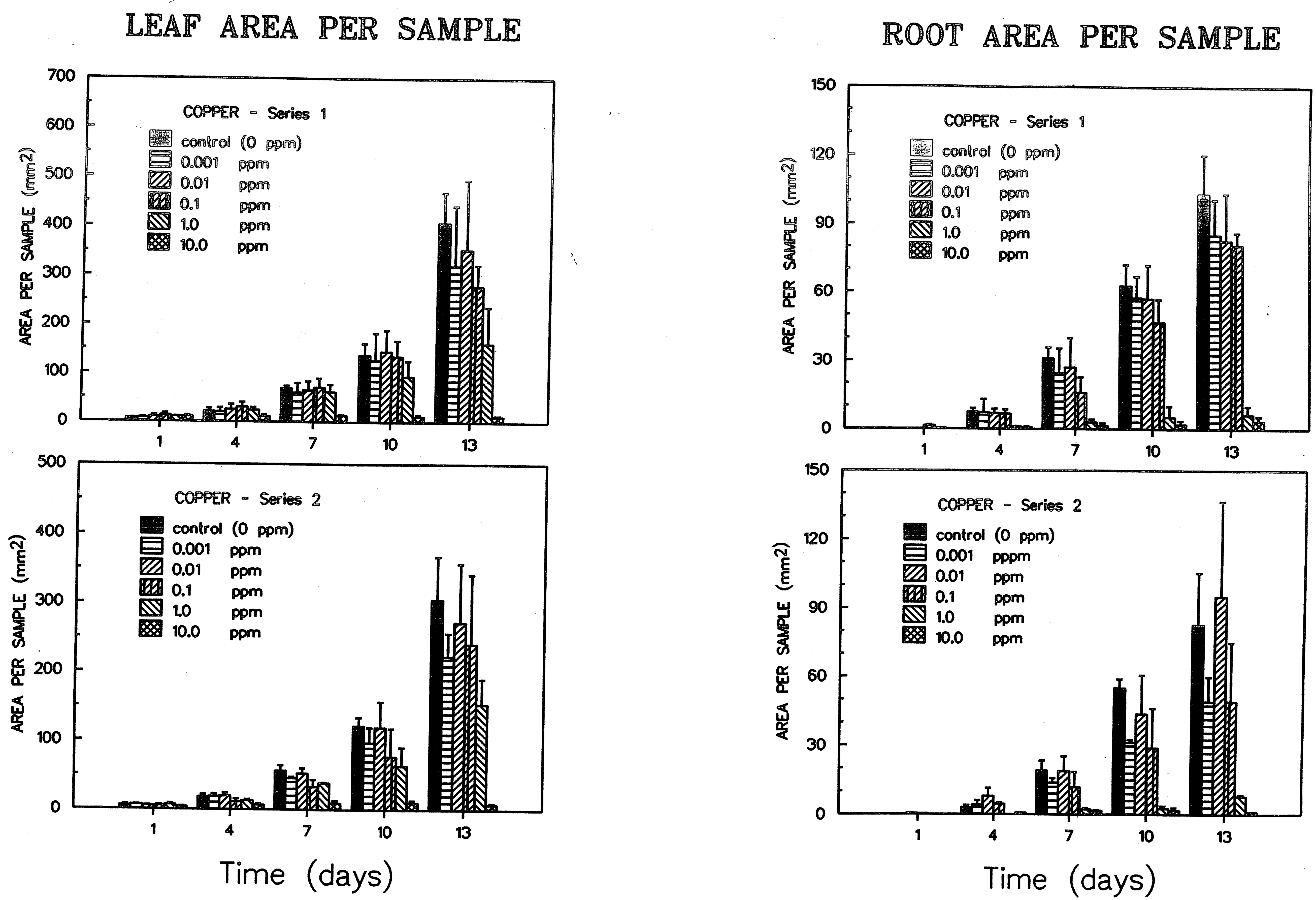


Figure 4: Leaf and root area of wild rice seedlings exposed to Copper treatments.

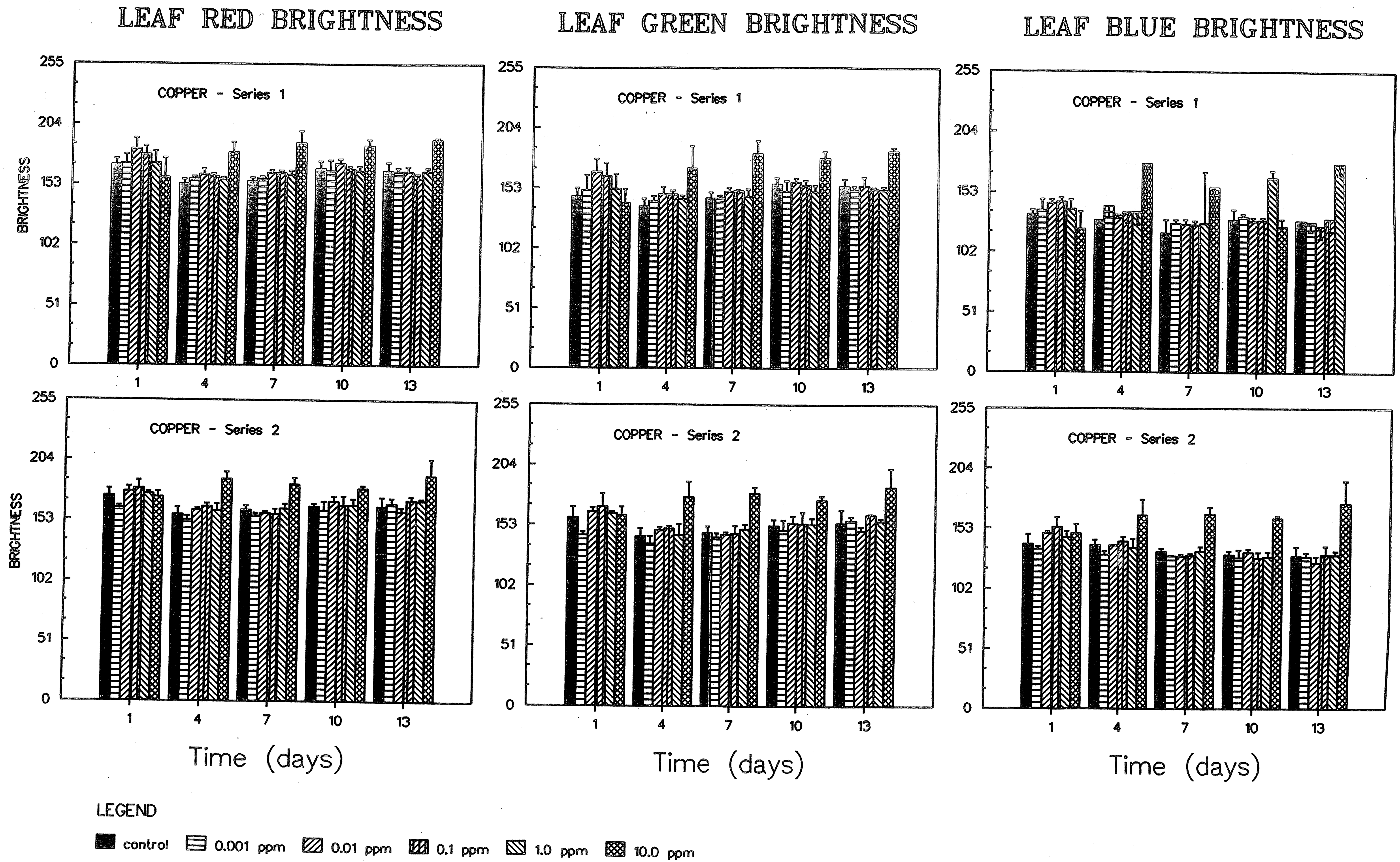


Figure 5: Leaf red, green and blue spectral values for wild rice seedlings exposed to Copper treatments.

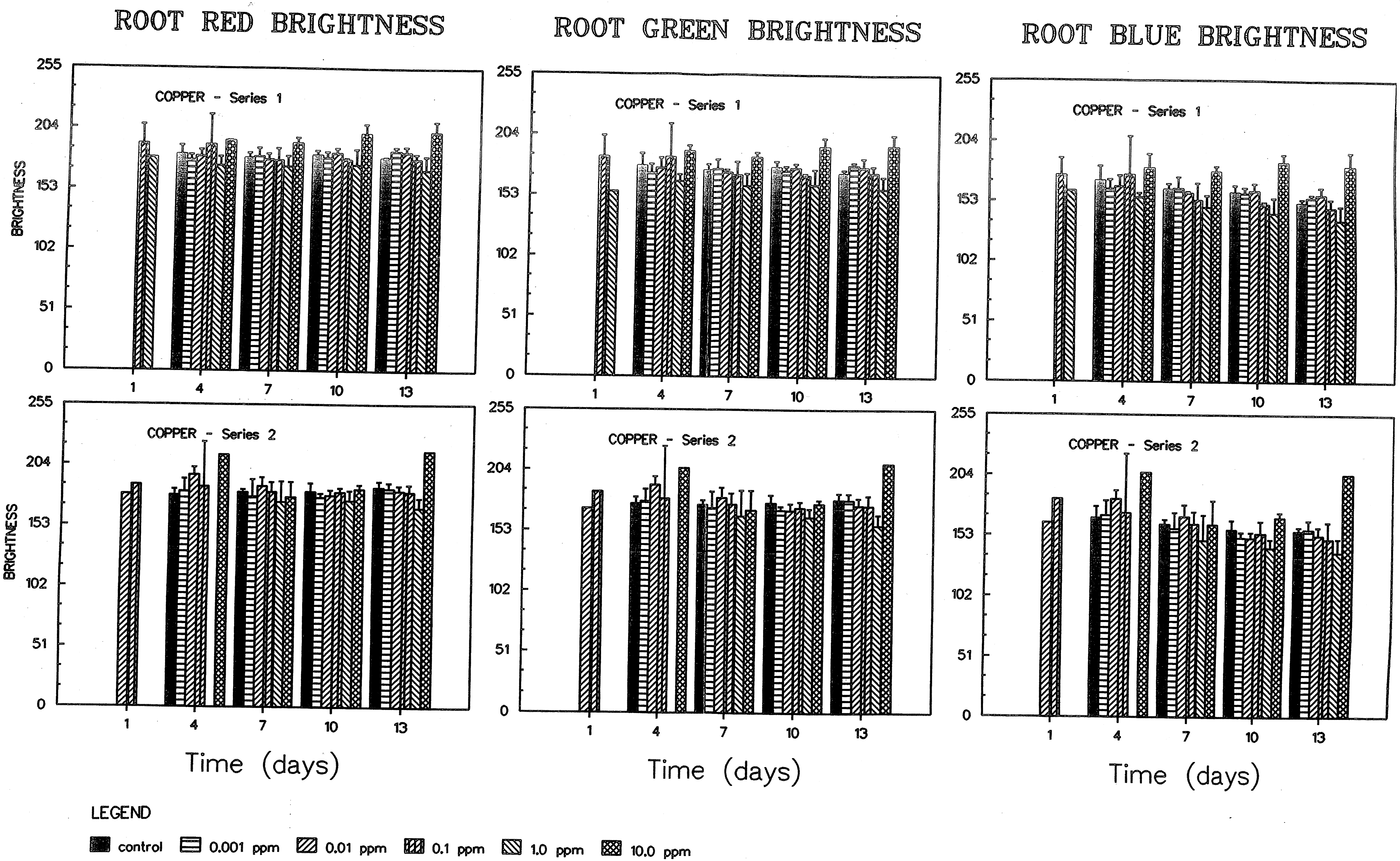


Figure 6: Root red, green and blue spectral values for wild rice seedlings exposed to Copper treatments.

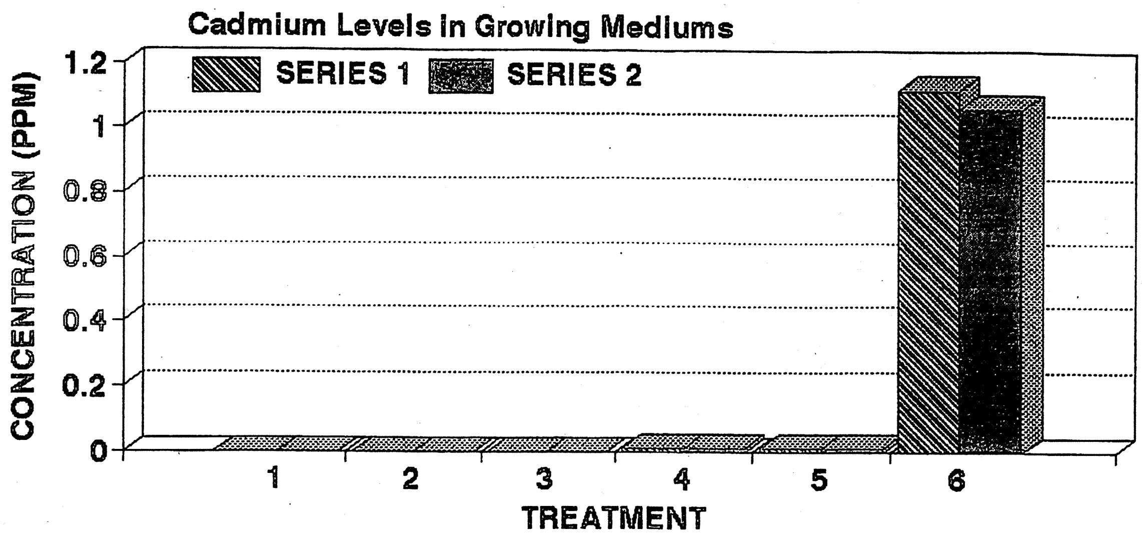
CADMIUM

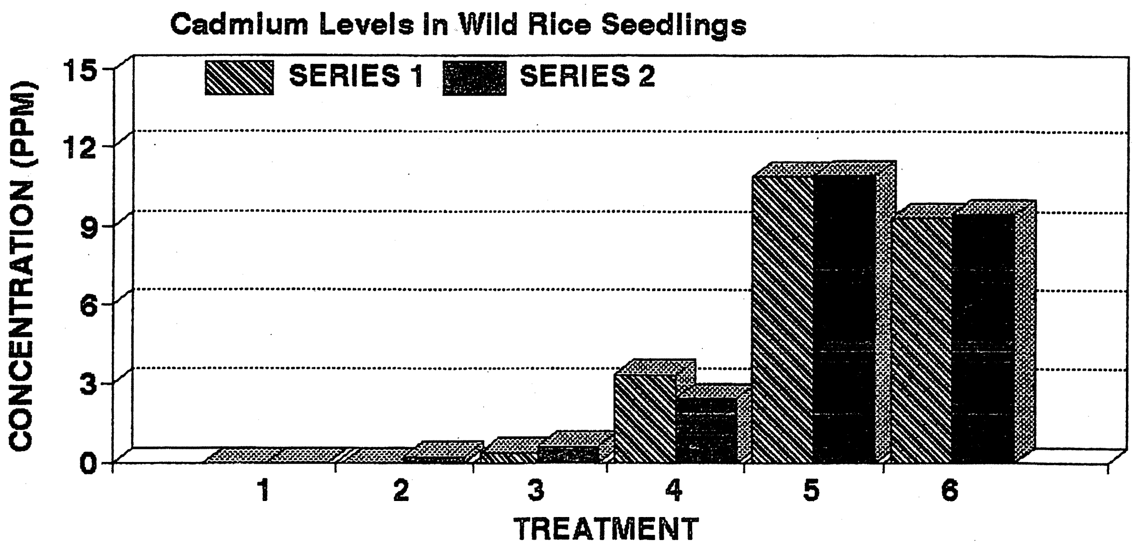
Plate 3 visually illustrates the differences that existed among the six Cd treatments. Shoot growth is noticeably reduced in Treatments 4 to 6. However, the most striking difference is the absence of root growth in Treatment 6. These differences were evident by the third sampling period.

Growth and colour differentiation among the treatments is shown by Figures 7 to 9, and statistically quantified in Table 7. As suggested by Plate 3, leaf area was statistically higher in Treatments 1 to 3. Treatment 6 had lower values for leaf area than any of the other treatments. In terms of root area, Treatment 6 had a significantly lower value than the other five treatments. Although there were some differences between treatments in leaf colour, these followed no distinct pattern and likely had little significance. Root colour brightness values, on the other hand, were significantly lower for Treatment 6, generally exhibiting a reddish tinge.

Levels in Growing Medium and Seedlings

Series 1 and Series 2 had similar Cd concentrations in both the growing medium and seedlings at the completion of the experiment (Table 3). Concentrations in the seedlings tended to increase as the levels in solution increased until Treatment 6 when the values remained similar to Treatment 5.





TREATMENT	SERIES	WATER ANALYSIS		TISSUE ANALYSIS
		MEAN	STD	MEAN
1	1	<0.004	n/a	<0.004
	2	<0.004	n/a	<0.004
2	1	<0.004	n/a	<0.004
	2	<0.004	n/a	0.19
3	1	<0.004	n/a	0.34
	2	<0.004	n/a	0.57
4	1	0.009	0.002	3.34
	2	0.009	0.001	2.45
5	1	0.010	0.001	10.88
	2	0.010	0.004	10.92
6	1	1.121	0.012	9.32
	2	1.062	0.082	9.43

Table 3: Cadmium detected in wild rice seedlings and growing mediums in ppm.

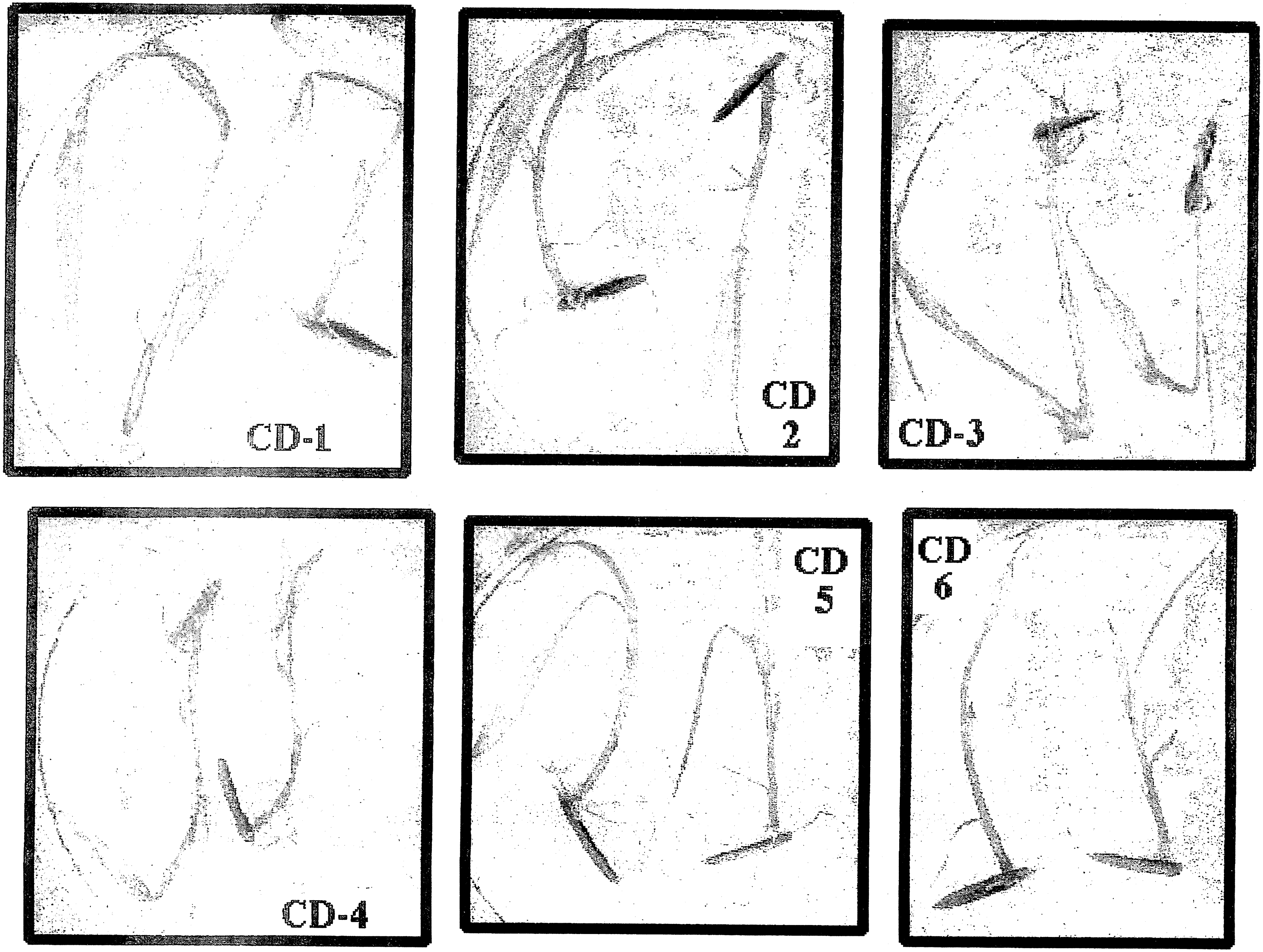


PLATE 3: Typical appearance of wild rice seedlings exposed to Cd treatments for 13 days.

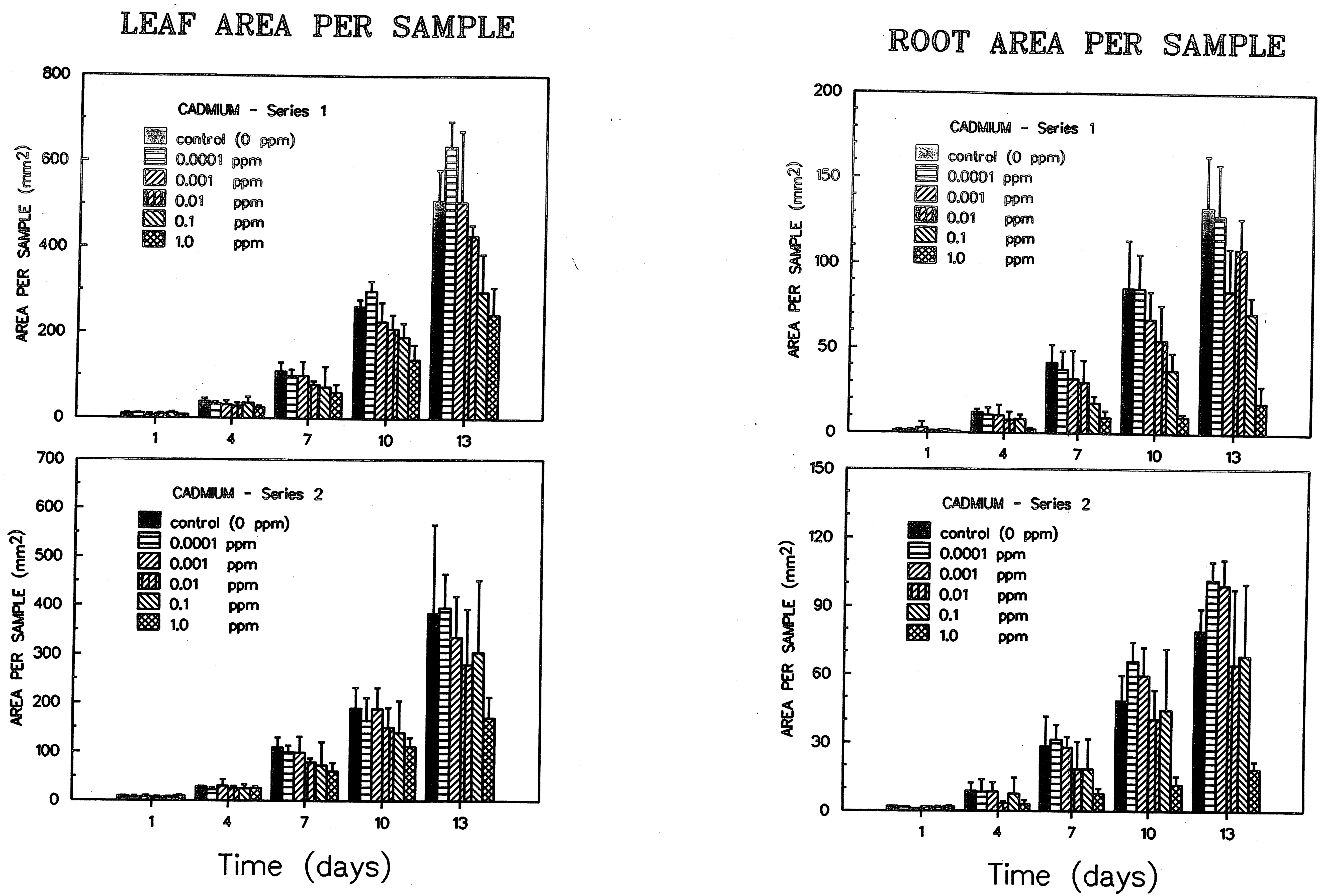


Figure 7: Leaf and root area of wild rice seedlings exposed to Cadmium treatments.

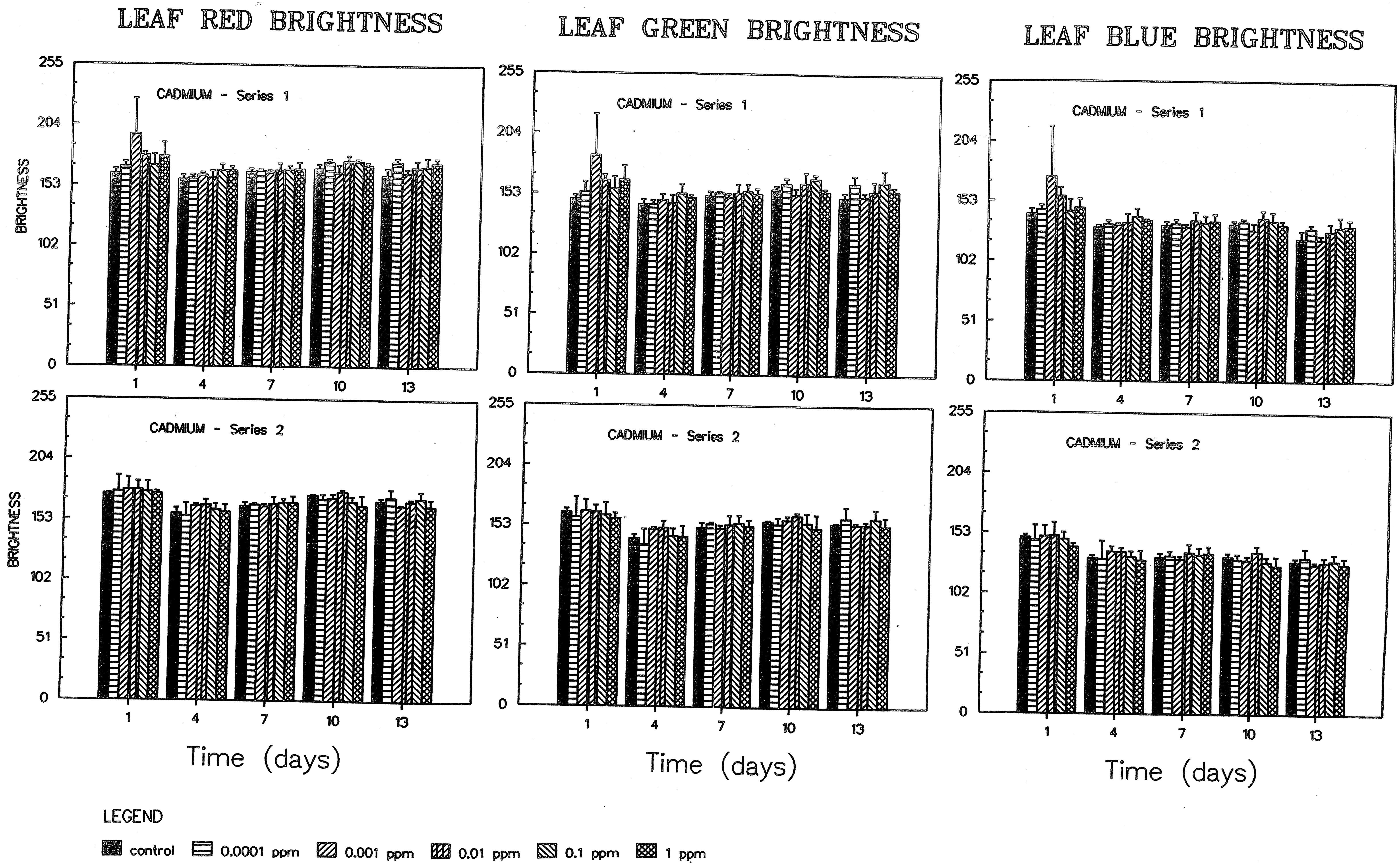


Figure 8: Leaf red, green and blue spectral values for wild rice seedlings exposed to Cadmium treatments.

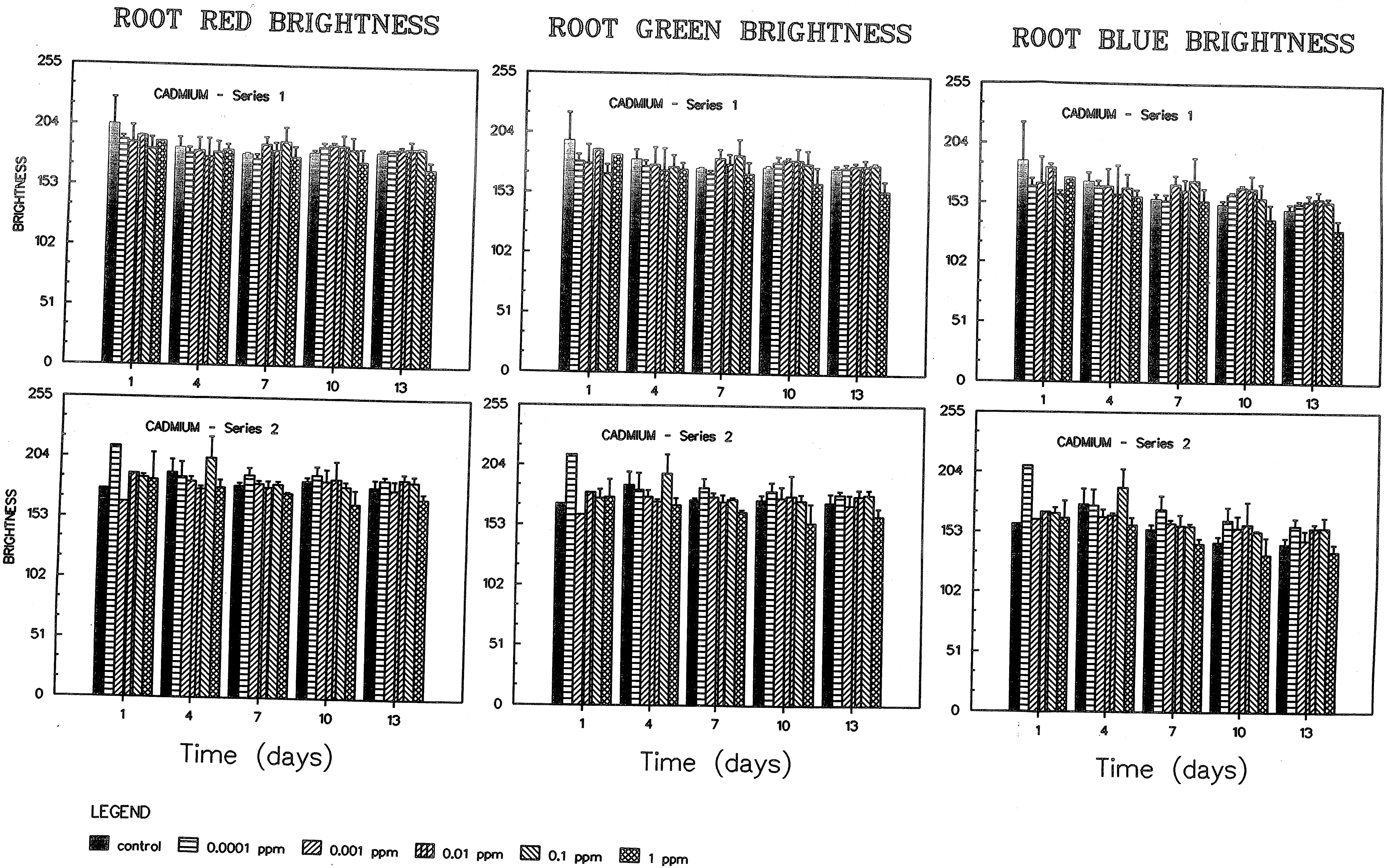


Figure 9: Root red, green and blue spectral values for wild rice seedlings exposed to Cadmium treatments.

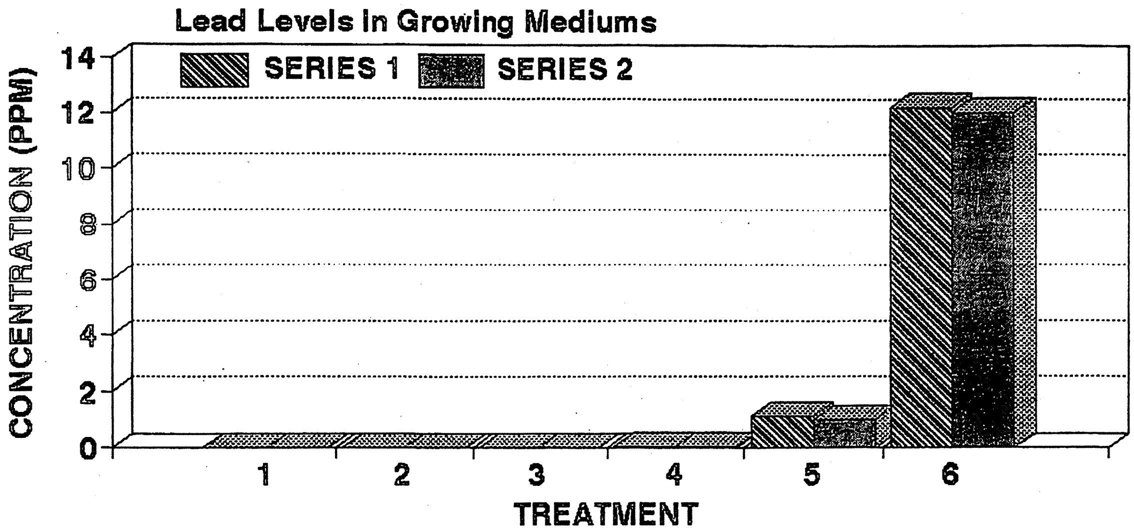
LEAD

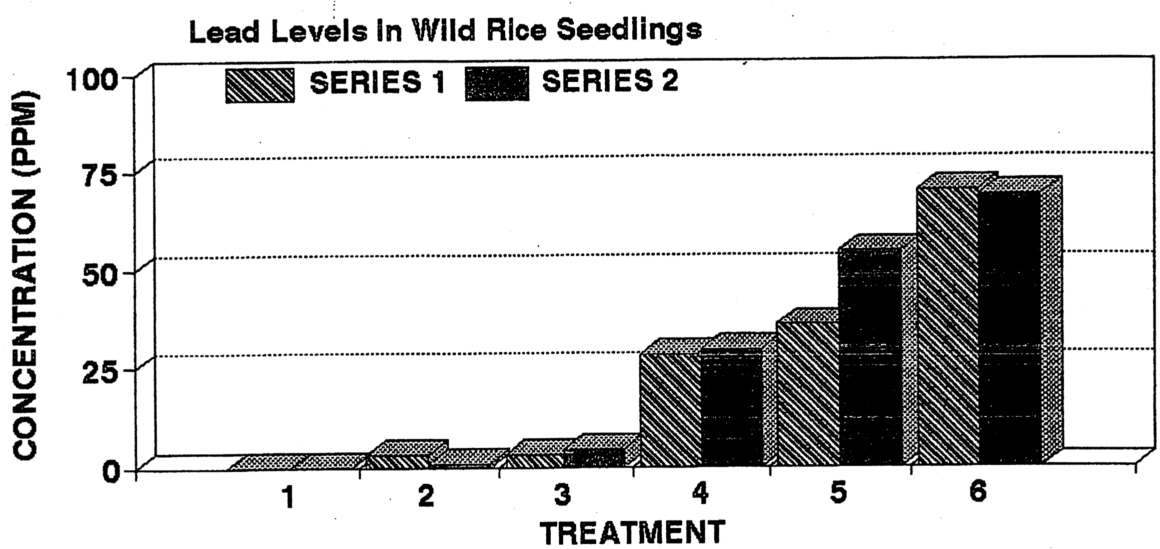
Visual differences among the six lead treatments are shown by Plate 4. As with Cd, Treatments 1 to 3 had greater shoot growth than Treatments 4 and 5. The seedlings were dead in Treatment 6. Root growth was absent from Treatment 5.

Growth performance and colour differentiation are shown by Figures 10 to 12 and statistically described in Table 7. Both leaf and root areas were statistically lower in Treatments 5 and 6 than Treatments 1 to 4. However, the absence of root growth in Treatment 5 did not distinguish it from Treatment 6 for this factor. Colour brightness values showed that Treatment 6 had significantly higher brightness values than the other treatments for green in leaves, and red, green and blue in roots. These trends were evident by the third sampling period.

Levels in Growing Medium and Seedlings

Concentrations of lead in the growing medium and the wild rice seedlings were similar for both series (Table 4). As with the other metals (except Cu), tissue concentrations increased as the solution concentrations increased.





TREATMENT	SERIES	WATER ANALYSIS		TISSUE ANALYSIS
		MEAN	STD	MEAN
1	1	<0.025	n/a	<0.025
	2	<0.025	n/a	<0.025
2	1	<0.025	n/a	2.965
	2	<0.025	n/a	0.649
3	1	<0.025	n/a	3.034
	2	<0.025	n/a	4.246
4	1	0.042	0.007	28.300
	2	0.031	0.001	29.316
5	1	1.137	0.032	35.771
	2	1.007	0.047	54.659
6	1	12.225	0.299	69.872
	2	12.048	0.486	68.720

Table 4: Lead detected in wild rice seedlings and growing mediums in ppm.

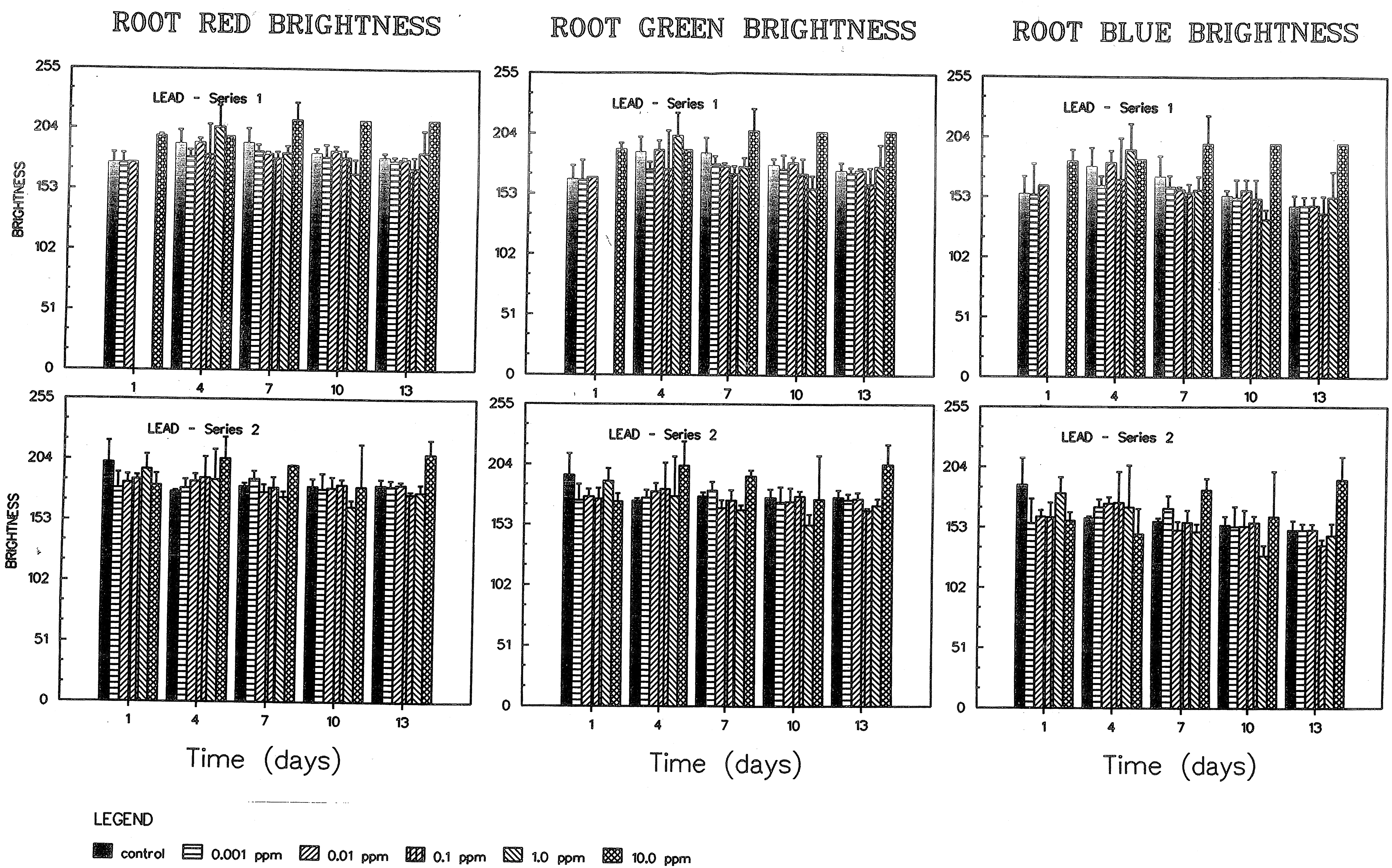


Figure 12: Root red, green and blue spectral values for wild rice seedlings exposed to Lead treatments.

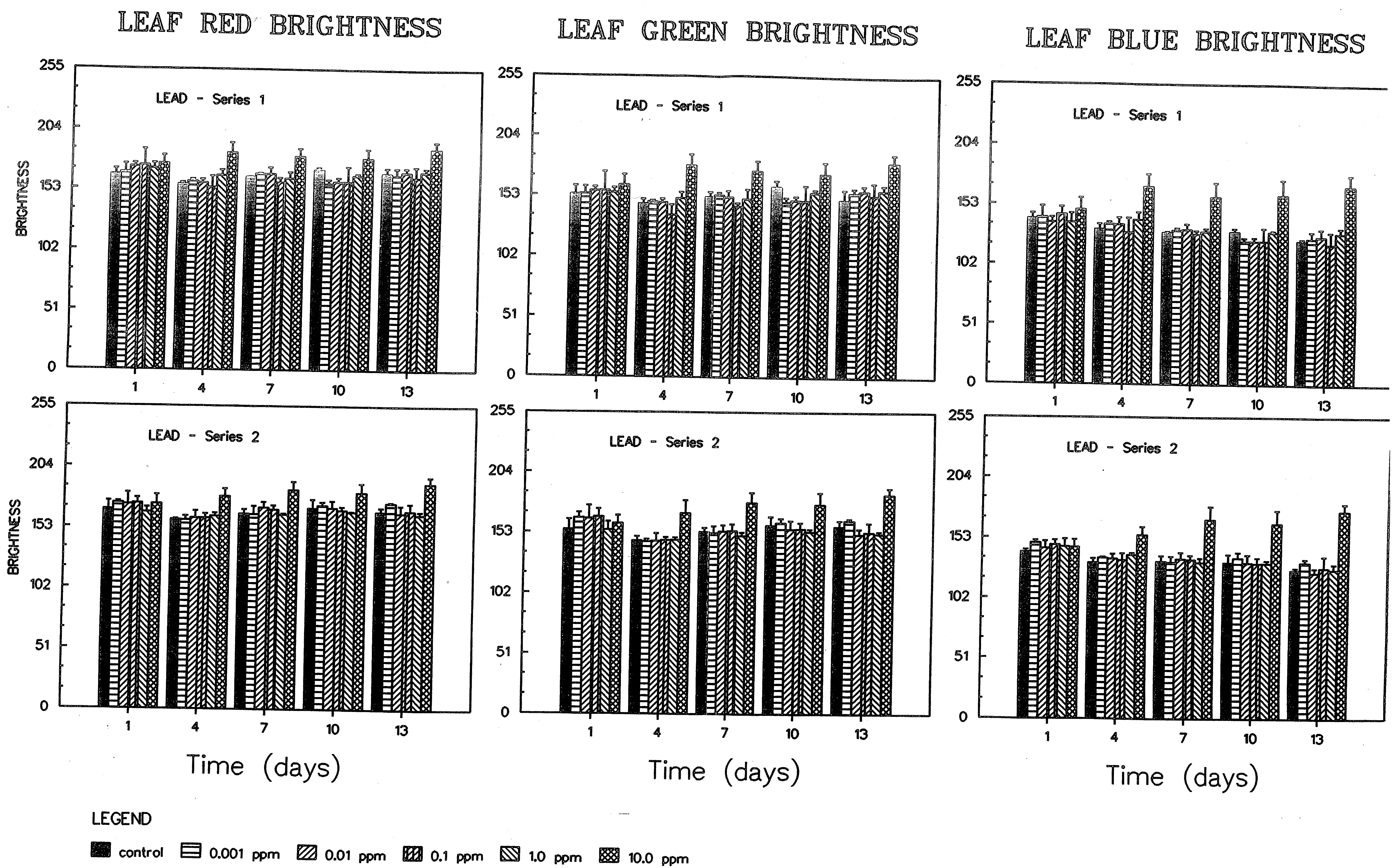


Figure 11: Leaf red, green and blue spectral values for wild rice seedlings exposed to Lead treatments.

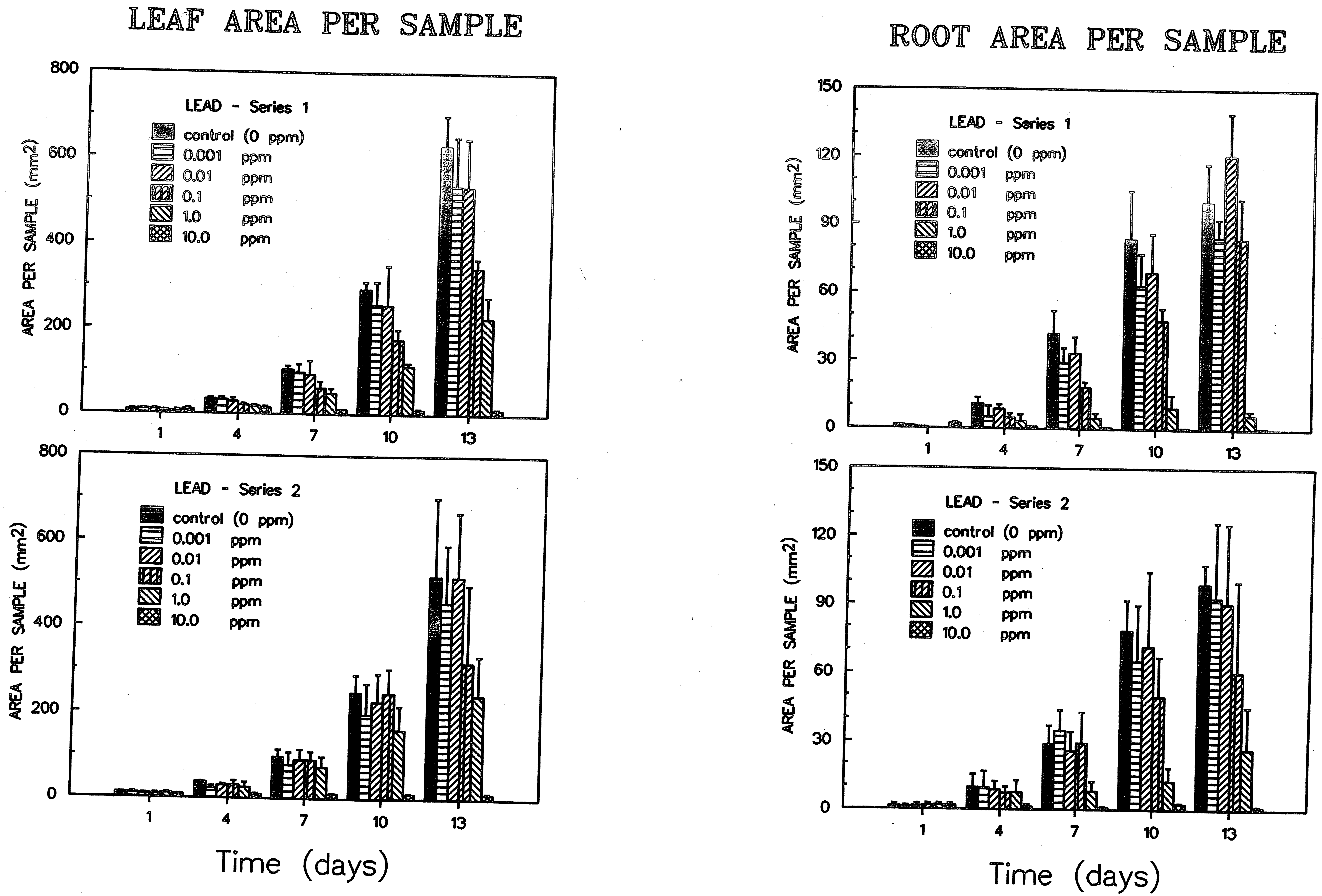


Figure 10: Leaf and root area of wild rice seedlings exposed to Lead treatments.

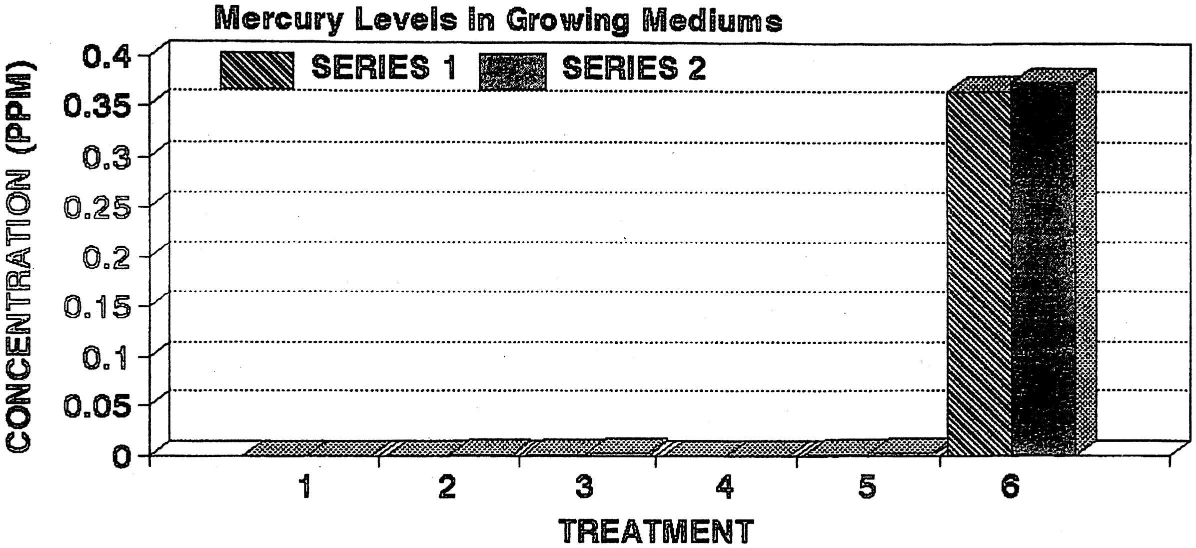
MERCURY

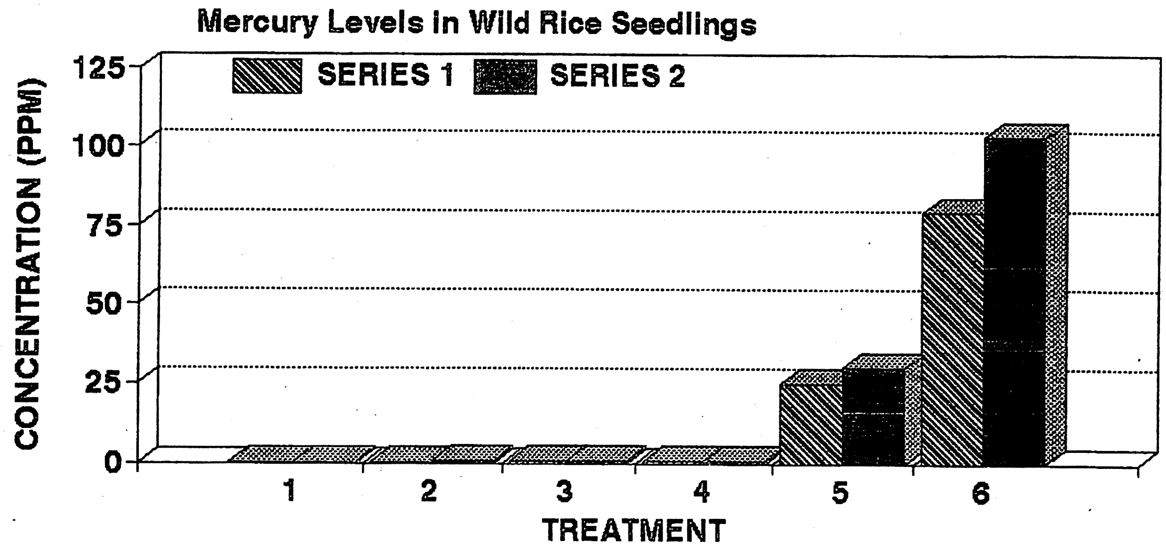
Plate 5 shows the visual appearance of the six mercury treatments. The most noticeable difference among the treatments was the reduced shoot growth and absence of root growth in Treatment 6. This trend was observable by the third sampling period.

Leaf and root areas are shown by Figure 13, and colour brightness values by Figures 14 and 15. Table 7 contains the results for between treatment LSD tests. Leaf area was significantly higher between Treatment 1 and all other treatments and significantly lower between Treatment 6 and all other treatments. Root area was significantly lower in Treatment 6 than all other treatments. Although some statistically significant paired differences occurred between the treatments for colour values, there were no definite patterns to the occurrence of these pairs.

Levels in Growing Medium and Seedlings

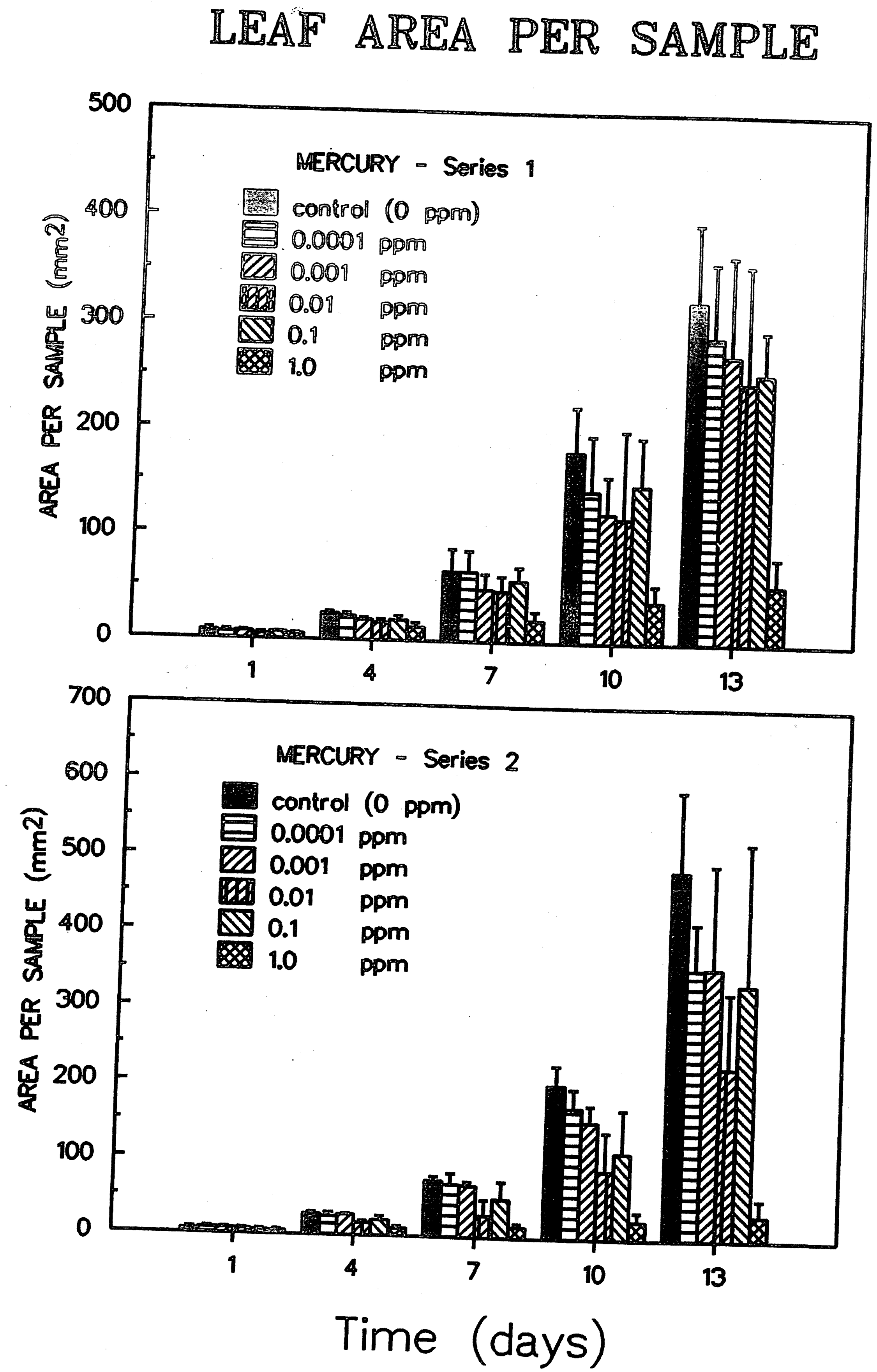
There seemed to be problems detecting the lower concentrations of Hg in Treatment 1 to 4 growing medium. The seedling concentrations, however showed a pattern of progressive increasing Hg concentration as the solution concentration increased (Table 5).





TREATMENT	SERIES	WATER ANALYSIS		TISSUE ANALYSIS
		MEAN	STD	MEAN
1	1	<0.00020	n/a	0.150
	2	<0.00020	n/a	0.330
2	1	<0.00020	n/a	0.230
	2	0.00075	n/a	0.480
3	1	0.00032	n/a	0.450
	2	0.00206	n/a	0.600
4	1	<0.00020	n/a	0.500
	2	<0.00020	n/a	0.500
5	1	0.00102	n/a	25.000
	2	0.00129	n/a	30.100
6	1	0.36568	n/a	79.200
	2	0.37468	n/a	103.000

Table 5: Mercury detected in wild rice seedlings and growing mediums in ppm.



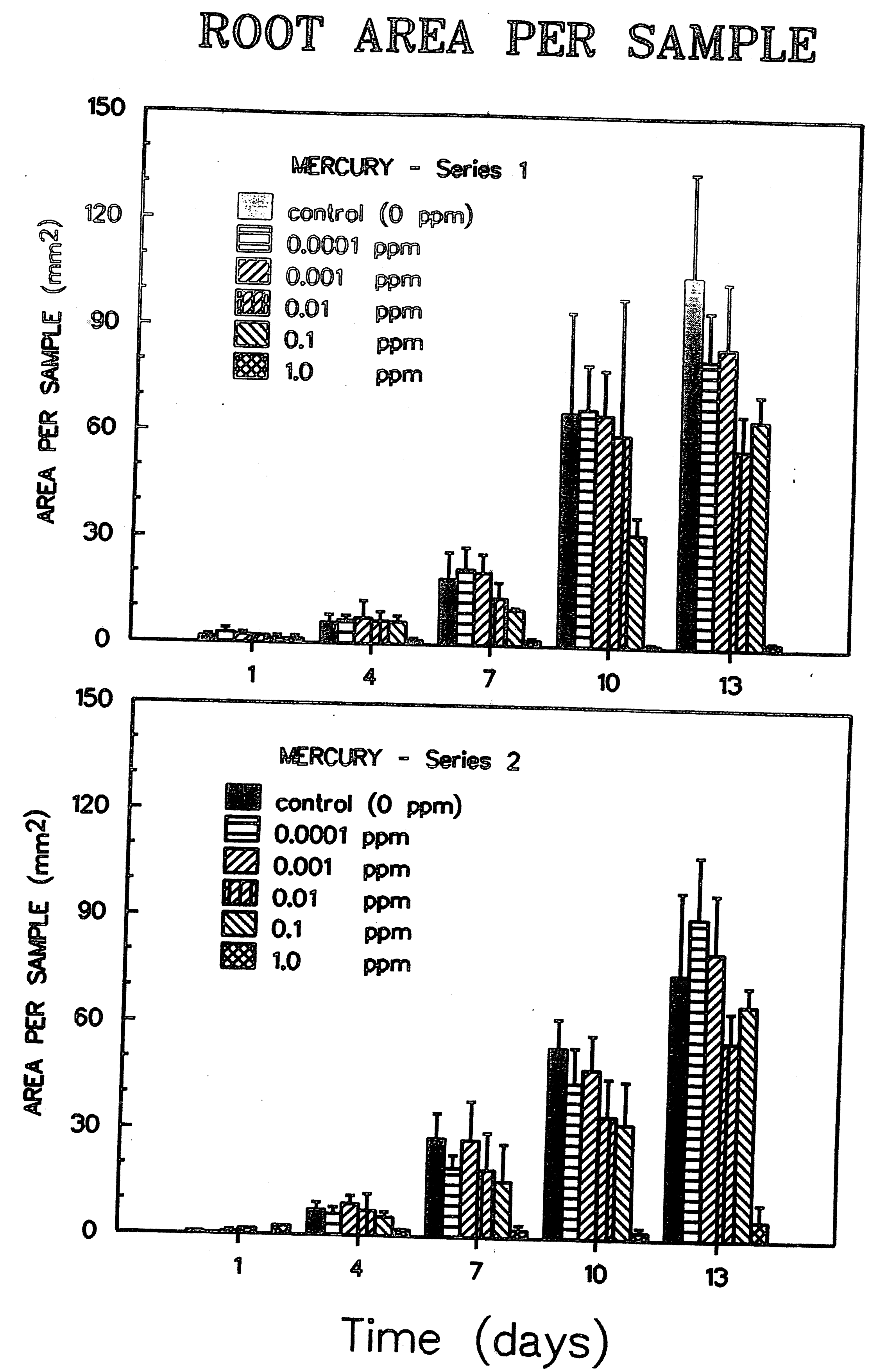


Figure 13: Leaf and root area of wild rice seedlings exposed to Mercury treatments.

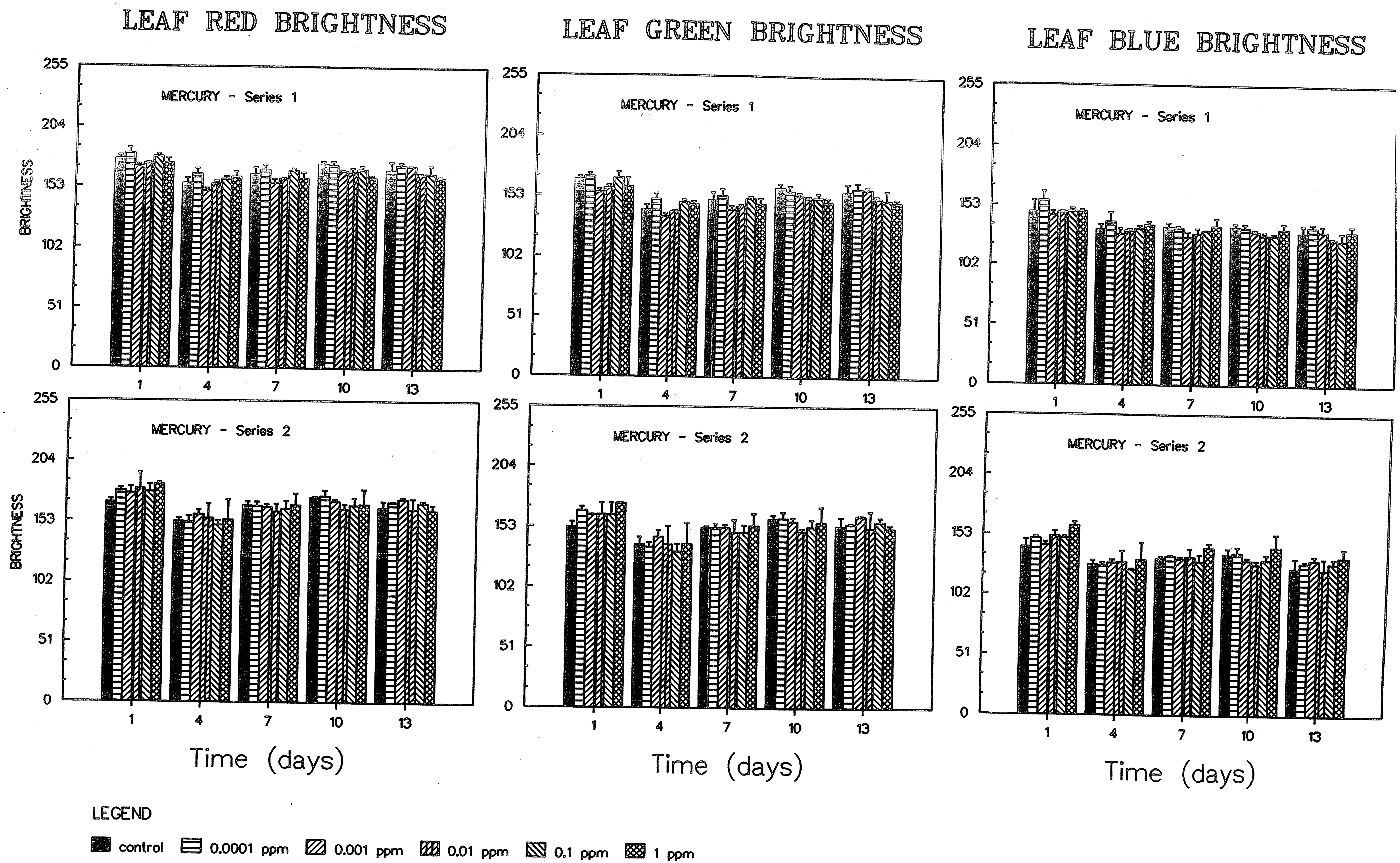


Figure 14: Leaf red, green and blue spectral values for wild rice seedlings exposed to Mercury treatments.

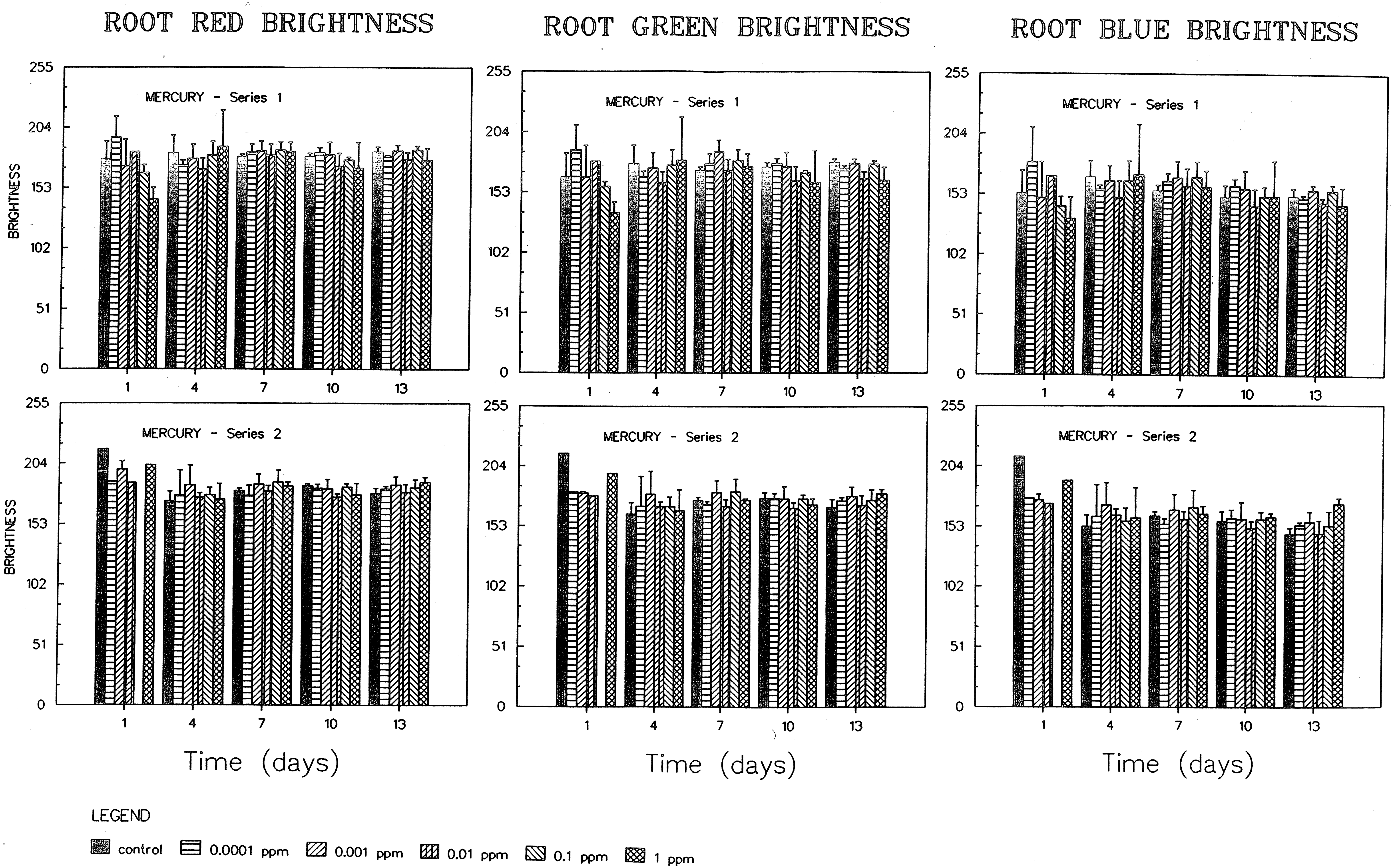
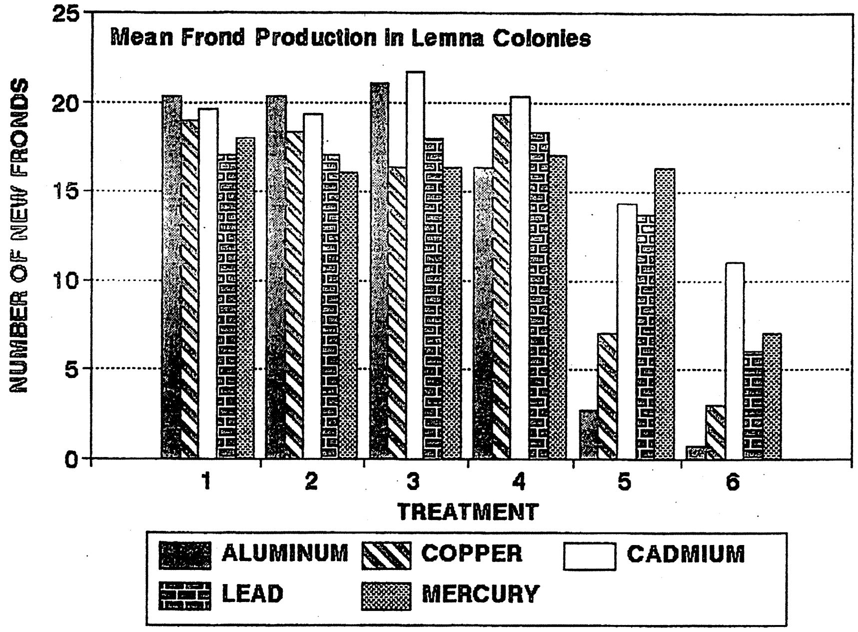


Figure 15: Root red, green and blue spectral values for wild rice seedlings exposed to Mercury treatments.

B. LEMNA

The effects of the metal treatments on Lemna are shown in Table 6. Declines in frond production started in Treatment 4 for Al, and Treatment 5 for Cu, Cd, Pb, and Hg. In all metals, frond production was greatly reduced in Treatment 6. The number of broken colonies and the presence of chlorosis followed this same pattern.



Mean Frond Production in Lemna Colonies

Element	Treatment 1	2	3	4	5	6
Aluminum	20.33	20.33	21.00	16.33	2.67	0.67
Copper	19.00	18.33	16.33	19.33	7.00	3.00
Cadmium	19.67	19.33	21.67	20.33	14.33	11.00
Lead	17.00	17.00	18.00	18.33	13.66	6.00
Mercury	18.00	16.00	16.33	17.00	16.33	7.00

Mean Number of Broken Lemna Colonies

Element	Treatment 1	2	3	4	5	6
Aluminum	0	0	0	2.00	3.67	0
Copper	0	0	0	0	9.00	0.67
Cadmium	0	0	0	0	0	6.33
Lead	0	0	0	0	2.33	0.67
Mercury	0	0.33	0.33	0	0.33	6.67

Presence (+) or Absence (-) of Chlorosis in Lemna

Element	Treatment 1	2	3	4	5	6
Aluminum	-	-	-	+	+	+
Copper	-	-	-	-	+	+
Cadmium	-	-	-	-	-	+
Lead	-	-	-	-	-	+
Mercury	-	-	-	-	+	+

Table 6: Response of Lemna to aluminum, copper, cadmium, lead and mercury treatments.

ALUMINUM

Treatment	Leaf Area	Leaf Red	Leaf Blue	Leaf Green	Root Area	Root Red	Root Blue	Root Green
1	a	n/a	a	n/a	a	a	a	a
2	a	n/a	a	n/a	a	ab	a	a
3	a	n/a	a	n/a	a	ab	ab	a
4	b	n/a	a	n/a	b	a	a	a
5	c	n/a	b	n/a	c	bc	bc	a
6	c	n/a	c	n/a	c	c	c	b

COPPER

Treatment	Leaf Area	Leaf Red	Leaf Blue	Leaf Green	Root Area	Root Red	Root Blue	Root Green
1	a	a	a	ab	a	a	a	a
2	bc	a	a	a	b	a	a	a
3	ab	a	a	ab	a	a	a	a
4	c	a	a	b	b	a	a	a
5	d	a	a	ab	c	b	b	b
6	e	b	b	c	c	c	c	c

CADMIUM

Treatment	Leaf Area	Leaf Red	Leaf Blue	Leaf Green	Root Area	Root Red	Root Blue	Root Green
1	a	a	ab	a	a	a	a	a
2	a	a	bc	ab	a	a	b	a
3	a	a	b	ab	ab	a	ab	a
4	b	a	c	ab	bc	a	ab	a
5	b	a	bc	b	c	a	b	a
6	c	a	bc	ab	d	b	c	b

LEAD

Treatment	Leaf Area	Leaf Red	Leaf Blue	Leaf Green	Root Area	Root Red	Root Blue	Root Green
1	a	n/a	n/a	a	a	a	a	a
2	bc	n/a	n/a	a	ab	a	a	a
3	ab	n/a	n/a	ab	a	a	a	a
4	c	n/a	n/a	b	b	a	a	a
5	d	n/a	n/a	b	c	a	a	a
6	e	n/a	n/a	c	c	b	b	b

MERCURY

Treatment	Leaf Area	Leaf Red	Leaf Blue	Leaf Green	Root Area	Root Red	Root Blue	Root Green
1	a	ab	ab	ab	a	ab	ab	ab
2	b	a	bc	a	ab	ab	ac	ab
3	b	acd	a	ab	a	b	c	a
4	c	bce	a	b	b	a	a	b
5	b	ade	a	ab	b	b	bc	ac
6	d	bd	c	ab	c	ab	bc	bc

Table 7: Statistically significant differences in area and colour between treatments. Treatments denoted by at least one common letter are statistically similar.

DISCUSSION

METAL TOXICITY EFFECTS ON WILD RICE

Plates 1 to 5, Figures 1 to 15, and Table 7 showed that the addition of heavy metals affected the growth of wild rice. In most cases, the effects were similar. Al, Cu, Cd, Pb, and Hg, at sufficient concentration, reduced the size of shoots and caused root production to cease. Mortality occurred at the highest concentrations tested of Al, Cu, and Pb. Changes in colour were also evident. Chlorosis occurred in leaves and roots at the higher treatment levels for Al, Cu and Pb. There was little noticeable increase in colour brightness values for Cd and Hg except for root values for Cd which actually declined with increasing amounts of Cd.

Of the observed effects, the cessation of root elongation is the most common result reported for other species from Al toxicity studies (Howeler and Cadavid, 1976; Pan et al, 1989; Kinraide et al, 1985). Root growth inhibition from Al may be a result of the inhibition of mitosis in the root tip (Naidoo et al, 1978) or a combination of a slowing of mitotic activity and rupture of epidermal root tissue. The other metals have been less studied for their effect on crops because they are relatively uncommon in field situations and there is no reason to select cultivars adapted to such conditions. Of the remainder, lead is a likely the most common contaminant and is known to be taken up by field crops (Bazzaz et al, 1974). Controlled growth of tomato, radish, bean and lettuce under conditions of elevated lead showed corresponding uptake into the roots (Tung and Temple, 1996), but the authors of this study did not mention any physical differences in the appearance of the plants. Wang (1986) did show that millet roots were impeded by effluent that contained Pb, Hg, Cu, and Cd, but the effluent also contained high levels of Zn, nitrates, phenols and oil, and so these results cannot be attributed solely to heavy metals. The main comparison available for heavy metal effects on wild rice with other plant species seems to be from duckweed. However, since Lemna is a floating rather than a rooted aquatic, caution should be exerted in the significance of such a comparison.

TOXICITY OF METALS - LEMNA VERSUS WILD RICE

Wang's (1990) EC50 values, (the value of the contaminant in solution at which duckweed had one half the growth of the control solution) was 0.2 ppm for Cd, 1.1 ppm for Cu, and 8.0 ppm for Pb. In the present experiment, statistically lower values occurred for Cd at a concentration of 0.01 ppm for leaves and 1.0 ppm for roots; for Cu at a concentration of 1.0 for leaves and roots; and for Pb at a concentration of 1.0 for leaves and roots. These concentrations (with the exception of leaf Cd where 1 ppm should be used) would approximate the LC50 values for wild rice. Published LC50 values for duckweed are not available for Al and Hg, but the results from this study (Table 6) would indicate that they occur at levels of 10 ppm for Al, and 1 ppm for Hg. With wild rice, statistically significant differences occurred between all treatments paired with Al at 10 ppm for leaves and roots, and with Hg at 1 ppm for leaves and roots.

The above comparisons suggest that wild rice has similar sensitivities as duckweed to metal concentrations, with the exception of Pb, where wild rice is considerably more sensitive. It is also noteworthy that duckweed exhibited comparable behaviour in the wild rice growth solution used in this study to the solution recommended by Standard Methods specifically for Lemna.

EFFECTIVENESS OF WILD RICE BIOASSAY

The results showed that wild rice was an effective assay for assessing the toxicity of contaminants. In addition to being at least as sensitive as duckweed to contaminants, it displayed a consistent response to the metal additions in both experimental series. The major drawback to wild rice is the large within treatment variation that occured. This variation however, seems to be comparable to other plants used for bioassays. The % coefficient of variation used by Wang (1986) to compare the effectiveness of test species for bioassays, was applied to wild rice. Using the mean and standard deviations of all control treatments, the coefficient of variation for wild rice was 36% (based on the results of the 10 time independent control treatment values - 162 ± 79, 158 ± 60, 166 ± 61, 146 ± 38, 132 ± 49, 118 ± 33, 162 ± 78, 137 ± 61, 146 ± 39, 153 ± 37). By comparison, Wang (1986) calculated the coefficient of variation for cucumber as 61%, lettuce, 33%, and millet, 64%.

The use of image analysis for leaf and root area measurements was also an efficient method to assess toxic effects on wild rice. It is highly accurate and permitted non-destructive measurements of the ongoing effects of the metals. Furthermore, and perhaps its most convincing characteristic, the images of the plants can be saved and reviewed if required.

REQUIRED DURATION OF BIOASSAYS

Although the bioassays were continued for 13 days after germination, the effects of the metals were evident earlier. Based on the results (Fig. 1 - 14), the recommended durations for the various elements are as follows: Al - 10 days, Cu - 7 days, Cd - 7 days, Pb - 7 days, and Hg -7 days.

TISSUE AND NUTRIENT CONCENTRATIONS

The results showed that metal concentrations in plant tissue increased with concentrations in solution. Similar luxury consumption of metals by wild rice was shown under field conditions by Lee and Stewart (1983). Therefore there is no doubt that if metals are present, they will be taken up by wild rice and will affect the growth performance of the plants. The levels used in the present study that affected wild rice were likely far greater than are naturally found. However, under contaminated conditions the levels were not inappropriate. Under acid conditions, Al concentrations can reach 4 ppm and toxicity from this element is a major problem for a variety of field crops (Blum, 1988). Shutes et al, (1993) reported the following levels of metals in a flood storage reservoir near London, England, as: Cd, 12.4 ppm; Cu, 220.1 ppm; Pb, 841.2 ppm. In other urban areas, lead levels have been reported to be high as 10,000 ppm (Linzon et al, 1976). For the specific case of a mine, typical pond water quality in an unoxidized tailing pond in northwestern Ontario had Pb and Cu levels of 0.11 ppm and 0.52 ppm respectively (Noranda Technology Centre, personal communication). These levels would be expected to be much elevated in the underlying sediment.

EXTRAPOLATION OF RICESTALK LAKE RESULTS TO MOLE LAKE

Previous studies have shown that there is substantial genetic heterogeneity among wild rice populations resulting in variations in such characteristics as plant height, tiller capacity, seed length and seed number (Counts and Lee, 1987; 1988). At least in their appearance in the field, the Ricestalk and Mole Lake plants are very different. Ricestalk has longer but fewer grains per panicle and has much less vegetative growth versus Mole Lake rice (personal observations). Since climate was the main factor influencing population variation in the above studies, these differences would be expected. However, what is important in this study is whether or not the response to metal concentrations would be similar. Counts and Lee (1988) showed that the local environment (mostly nutrient levels) explained 20 - 30% of the differences in wild rice productivity and morphology among populations, versus 50 to 66 % for climate. Therefore although nutrients generally have a lesser role in the selection pressure on wild rice populations, such selection can occur. In fact since Al levels were considerably higher in Ricestalk vs. Mole Lake, this could indicate that Ricestalk is exposed to higher Al levels and therefore possibly more tolerant of Al levels than Mole Lake. The only conclusive way to determine the effect on Mole Lake seed is to test it with the metal additions.

CONCLUSIONS AND RECOMMENDATIONS

Based on the results of this study, the following conclusions can be made:

1. wild rice is adversely affected by elevated levels of heavy metals which cause reduced shoot and root growth and chlorosis of leaves and roots. At sufficiently high levels of the metals, mortality of the exposed seedlings occurs.

2. concentrations (ppm) at which adverse affects are initiated in wild rice are: Al, 10; Cu, 1.0; Cd, 0.01 (leaves), 1.0 (roots); Pb, 1.0; and Hg, 1.0. These adverse effects are evident by day 7 after exposure.

3. a bioassay, which uses image analysis to measure leaf and root area, can accurately quantify the effects of the metal contaminants.

Although these findings are conclusive, additional investigations are required to extend them to the specific case of the Mole Lake seed. In this regard the following recommendations are made:

a. tests similar to those performed in this experiment be repeated for Mole Lake seed. Some variations in the duration of the tests could be made to reduce the amount of data to be processed. Perhaps a combination of more series of experiments for a shorter exposure time would be better.

b. the natural variation for seedling size and chemical composition that exists within the Mole Lake population should be quantified by growing test groups in the control nutrient solution prior to initiating the exposure experiments. These results may influence the sample size required.

c. the precision of image analysis of leaves and roots should be determined by quantifying the effect of repeated scans of the same plant, and variation among analysts of the same images.

This study developed a bioassay that successfully demonstrated the effects of elevated levels of metals on wild rice. Image analysis measured and quantified these effects which were primarily reduced shoot and root growth.

REFERENCES

Aiken, S.G., Lee, P.F., and J.M. Stewart 1988. Wild Rice In Canada. NC Press Ltd., Toronto.

Bazzaz, F.A., Rolfe, G.L., and P. Windle. 1974. Differing sensitivity of corn and soybean photosynthesis and transpiration to lead contamination. J. Environ. qual. 3:156-158.

Beck, D.R.M. 1994. Wild rice in Menominee history. Report prepared for Mole Lake Indian Band.

Blum, A. 1988. Plant Breeding for Stress Environments. CRC press.Chambliss, C.E. 1940. The botany and history of Zizania aquatica L. ("Wild Rice"). J. Wash. Acad. Sci. 30:185-205.

Chapin, F.S., Follett, J., and K.F. O'Connor. 1982. Growth, phosphate absorption, and phosphorus chemical fractions in two Chionochloa species. Oecologia 79:96-105.

Counts, R.L. and P.F. Lee. 1988. Patterns of variation in Ontario wild rice (Zizania aquatica L.) 2.Differential responses of populations to varying cultivation conditions. Aquat. Bot. 31:23-36.

Counts, R.L. and P.F. Lee. 1990. Patterns of variations in Ontario wild rice (Zizania aquatica L.). 4.Relative contributions of some physical, edaphic, and biotic environmental factors to in situ variation within and among populations. Aquat. Bot. 36:193-205.

Counts, R.L. and P.F. Lee. 1991. Germination and early seedling growth in some northern wild rice Zizania palustris) populations differing in seed size. Can. J. Bot. 69:689-696.

Elakovich, S.D. and J.W. Wooten. 1991. Allelopathic potential of sixteen aquatic and wetland plants. J. Aquat. Plant Manage. 27:84-89.

Davies, M.S. and R.W. Snaydon. 1974. Physiological differences among populations of Anthoxanthum odoratum L. collected from the park grass experiment, Rothamsted. III. Response to phosphate. J.Appl.Ecol. 11:699-704.

Day, W.R. and P.F. Lee. 1989. Ecological relationships of wild rice, Zizania aquatica L. 8.Classification of sediments. Can. J. Bot. 67:1381-1386.

Day, W.R. and P.F. Lee. 1990. Mineral deficiencies of wild rice (Zizania aquatica L.) grown in organic-flocculent sediments. J. Aquat. Plant Manage. 28:84-88.

Devine, T.E. 1982. Genetic fitting of plants to problem soils. In M.N. Christiansen and C.F. Lewis (ed.) Breeding plants for less favorable environments. Wiley, New York.

Dore, W.G. 1969. Wild Rice. Can. Dept. of Agric. Pub. No. 1393, Ottawa.

Fyles, F. 1920. Wild Rice. Bull. no. 2, 2nd Series. Can. Dept. Agric., Ottawa.

Howeler, R.H. and L.F. Cadavid. 1976. Screening of rice cultivars for tolerance to Al-toxicity in nutrient solution as compared with a field screening method. Agron. J. 68:551-555.

Keenan, T. and P.F. Lee. 1987. Ecological relationships of wild rice, Zizania aquatica L. 7.Sediment nutrient depletion following introduction of wild rice to a shallow Boreal lake. Can. J. Bot. 66:236-241.

Kinraide, T.B., Arnold, R.C., and V.C. Balizar. 1985. A rapid assay for aluminum phytoxicity of submicromolar concentrations. Physiol. Plant. 65:245-250.

Lee, P.F. 1979. Biological, chemical and physical relationships of wild rice, Zizania aquatica L. in northwestern Ontario and northeastern Minnesota. Ph.D. thesis, Univ. of Manitoba.

Lee, P.F. 1986. Ecological relationships of wild rice, Zizania aquatica L. 4.Environmental regions within a wild rice lake. Can. J. Bot. 64: 2037-2044.

Lee, P.F. and J.M. Stewart. 1981. Ecological relationships of wild rice, Zizania aquatica L. 1.A model for among-site growth. Can. J. Bot. 59:2140-2151.

Lee, P.F. and J.M. Stewart. 1983. Ecological relationships of wild rice, Zizania aquatica L. 2.Sediment-plant tissue nutrient relationships. Can. J. Bot. 61:1775-1784.

Lee, P.F. 1986. Ecological relationships of wild rice, Zizania aquatica L. 4.Environmental regions within a wild rice lake. Can. J. Bot. 64: 2037-2044.

Linzon, S.N., Chai, B.L., Temple, P.J., Pearson, R.G., and M.L. Shutes, R.B., Ellis, J.B., Revitt, D.M., and T.T. Zhang. The use of Typha latifolia for heavy metal pollution control in urban wetlands. (IN) Constructed Wetlands for Water Quality Improvement, G.A. Moshiri, editor, CRC Press.

Moyle, J.B. 1944. Wild rice in Minnesota. J. Wildl. Man. 8(3):177-184.

Malvick, D.K. and J.A. Percich. 1993. Hydroponic culture of wild rice (Zizania palustris L.) and its application to studies of silcon nutrition and fungal brown spot disease. Can. J. Plant Sci. 73:969-975.

Naidoo, G., Stewart, J.M., and R.J. Lewis. 1978. Accumulation sites of Al in snap bean and cotton roots. Agron. J. 70:489-492.

Ottman, M.J. and H. Timm. 1984. Measurement of viable plant roots with the image analyzing computer. Agron. J. 76:1018-1020.

Pan, W.L, Hopkins, A.G., and W.A. Jackson. 1989. Aluminum inhibition of short lateral branches of Glycine max and reversal by exogernous cytokinin. Plant Soil 120:1-9.

Pip, E. and W. Paulishyn. 1971. The ecology and distribution of Promenetus exacuous Say in southern Manitoba. Can. J. Zool. 49:367-372.

Smith. 1976. Lead contamination of urban soils and vegetation by emissions from secondary lead industries. J. Air Pollut. Control Assoc. 26:650-654.

Snaydon, R.W. and A.D. Bradshaw. 1961. Differential responses to calcium within the species Festuca ovina L. New Phytol. 60:219-234.

Snaydon, R.W. and A.D. Bradshaw. 1969. Differences between natural populations of Trifolium repens L. in response to mineral nutrients. II. Calcium, magnesium, and potassium. J.App.Ecol. 6:185-202.

Steeves, T.A. 1952. Wild rice - Indian food and a modern delicacy. Econ. Bot. 6:107-142.

Sutton, D.L. and K.M. Porter. 1989. Influence of allelochemicals and aqueous plant extracts on growth of duckweeds. J. Aquat. Plant Manage. 24:16-20.

Taraldsen, J.E. and T.J. Norberg-King. 1990. New method for determining effluent toxicity using duckweed (Lemna minor). Environ. Toxicol. Chem. 9:761-767.

Tung, G. and P.J. Temple. 1996. Histochemical detection of lead in plant tissue. Environ. Tox. Chem. 15:906-915.

Vicario, B.T. and E.H. Halstead. 1968. Progress report on wild rice research. Univ. of Sask., Dept. of Soil Science.

Wang, W. 1986. Comparative toxicology of phenolic compounds using root elongation method. Environ. Toxicol. Chem. 5:891-896.

Wang, W. 1987. Root elongation method for toxicity testing of organic and inorganic pollutants. Environ. Toxicol. Chem. 6:409-414.

Wang, W. 1990. Toxicity testing and biomonitoring using aquatic plants. Aquaphyte 10(2), Centre for Aquatic Plants, Univ. of Fla.

Wang, W., and J.M. Williams. 1990. The use of phytotoxicity tests (common Duckweed, Cabbage, and Millet) for determining effluent toxicity. Environ. Monitoring and Assessment 14: 45-58.

APPENDIX 1

CHAIN OF CUSTODY RECORD

ALL PERSONS INVOLVED IN THE COLLECTION, TRANSPORTATION, ANALYSIS, STORAGE AND HANDLING OF THESE SAMPLES SHOULD READ THE FOLLOWING INSTRUCTIONS CAREFULLY.

Test results for these samples will be used in litigation. Samples must be accounted for at all times prior to analysis.

All persons involved in the collection, transportation, handling, analysis or storage of these samples **MUST** sign and date the times in which they were in possession of/or responsible for said samples on the accompanying "Chain of Custody Record." Your signature is your acknowledgement that you were in possession of/or responsible for the named samples for the time period designated. Before accepting possession or responsibility for the listed samples be certain that the sample numbers on the sheet that you sign, correspond to the sample numbers of the specimens you receive. Only sign for specimens that you receive. All days and times **MUST** be accounted for, with no gaps in dates or times. The collector should make the first entry.

Prior to and during analysis, samples should be stored in security areas to avoid tampering.

Mercury samples must be stored at 4°C and analyzed within 28 days of the date of collection. Lead, cadmium, aluminum and copper samples must be analyzed within 6 months of the collection date.

Please print clearly and include full addresses where applicable. Signed sheets should be returned with the sample analyses to the sender at the address below.

Thank you for your cooperation in this matter.

Dr. Peter Lee
c/o Lakehead University
955 Oliver Road
Thunder Bay, Ontario
CANADA
P7B 5E1

If you have any questions or problems please call:

Dr. Peter Lee 1-807-343-8662

6

CHAIN OF CUSTODY RECORD

Do not sign this form until you have read the attached page.

SAMPLE NUMBER(S) AL-1-1, AL-1-2, AL-1-3
AL-2-1, AL-2-2, AL-2-3

SAMPLE TYPE WATER

DATE RECEIVED (D.M.Y.)	TIME RECEIVED (AM/PM)	INSTITUTION/ADDRESS	FULL NAME	SIGNATURE
JULY 15. 96	8:40 PM	Lakehead University	PAULA HUGHES	Paula Hughes
July 16/96	10:00 am	Enviro Lab.	Johane Joncas	Johane J.
18/7/96	10:40 am	Inst. Lab. L.U.	Ain Raitsakas	[illegible]

SAMPLE ANALYSIS REQUEST SHEET

Please analyze the samples listed on page 2 of the SAMPLE ANALYSIS REQUEST SHEET for the metals indicated with a circled "YES" below.

METAL	*ANALYZE ?*	
Aluminum	*YES*	*NO*
Cadmium	*YES*	*NO*
Copper	*YES*	*NO*
Lead	*YES*	*NO*
Mercury	*YES*	*NO*

SAMPLE ANALYSIS REQUEST SHEET

The following questionnaire must be completed and signed just prior to analysis by a laboratory representative in charge of the sample.

Answer all questions, circling "YES" or "NO" where required. Answer all questions in INK.

1. 1a.	*Is the condition of the samples satisfactory?* *If NO, list samples that are unsatisfactory. Explain why. Notify the sender immediately.*	YES	NO
2. 2a.	***Are the seals intact?*** ***If NO, list seals that are broken and notify the sender immediately.***	YES	NO
3. 3a.	***Do the seals correspond to the bottle labels?*** ***If NO, list seals that are inconsistent and notify the sender immediately.***	YES	NO
4. 4a.	***Have you received all of the samples listed on the attached sheet?*** ***If NO, list samples that you are missing and notify the sender immediately.***	YES	NO

I hereby certify that I have checked all seals, containers and labels, that I have completed this form to the best of my ability, and that all information given is true.

Name: AIN RAITSAUKAS

Signature:

Date: 19/4/96

PROCEEDINGS OF THE WILD RICE RESEARCH & MANAGEMENT CONFERENCE

July 7-8, 1999

Carlton, Minnesota

THE ECOLOGY OF "WILD" WILD RICE (*ZIZANIA PALUSTRIS* VAR. *PALUSTRIS*) IN THE KAKAGON SLOUGHS, A RIVERINE WETLAND ON LAKE SUPERIOR

J. Meeker

ABSTRACT

The Kakagon Sloughs are situated on the southern shore of Lake Superior and are unique among the lake's wetlands in their wild rice dominance. In this study, the importance of water level fluctuations for wetland dynamics in Lake Superior was demonstrated for both the region and within the Kakagon. Regionally, mean wetland species richness was greatest along shoreline elevations and declined toward the highest and lowest elevations sampled. In the Kakagon, the vegetational response to water level fluctuations between 1986 and 1989 demonstrated that drawdown years are important in maintaining long-term wild rice abundance by allowing this annual species to rapidly re-colonize areas that were too deep for most aquatic species during the high water years.

At any location in the Kakagon, both channel morphometry and water depth greatly influence the physical environment that developing wild rice plants encounter after germination. In addition, the wild rice plants themselves modify the local environment and greatly influence the sedimentation regime. The data suggest that in many locations along the riverine wetland, the early growth stages of wild rice (the submersed and floating leaf stages) act as filters and trap sediment that then provides the nutrients necessary for the later demanding stages of stem elongation and grain development.

Wild rice productivity was greatest along riverine habitat and at moderate depths in locations just inside the vegetation-open water interface. Most wild rice mortality takes place early on during the submerged leaf stage, as plants are presumably dislodged from the sediment. At the individual plant level, experimental data suggested that there may be a threshold weight for individual plants, below which wild rice mortality is increased.

INTRODUCTION

Wild rice (*Zizania palustris* L. var. *palustris*) is a native North American grain that was once abundant across the "wild rice district" of northeastern Minnesota, northern Wisconsin, and southern Ontario (Jenks 1901). Even though wild rice is now being restored to original levels in many lakes throughout the region (David, this volume), research and restoration on riverine wild rice is less well developed, although it once dominated the shallow margins of gently flowing streams and rivers. In this paper, I will: 1) summarize the taxonomy of wild rice; 2) describe the phenology and life history of wild rice relative to my dissertation research in the riverine habitat of the Kakagon Sloughs; and 3) discuss the ecology of this riverine species based on what is known in the literature and my work in the Kakagon.

THE KAKAGON SITE

The Kakagon Sloughs and their associated wetlands lie on the shores of Lake Superior in northern Wisconsin and are recognized as a National Natural Landmark. The steward of this productive wetland complex is the Bad River Band of Lake Superior Chippewa, who, for centuries, have harvested wild rice from these waters. The research reported here is a response to the Bad River Band's concern for the long-term health of this wetland and the recognized void of information on the ecology of northern wild rice in riverine habitat.

The Kakagon Sloughs are a part of the Lake Superior lowland province and are directly influenced by lakewide water level changes. In addition, this wetland experiences considerable short-term water level fluctuations due to the seiche activity associated with Chequamegon Bay. The Kakagon Sloughs are also unique among Lake

Superior's wetlands in their wild rice dominance. As my dissertation research suggests (Meeker 1993, 1996), both the estuarial characteristics of the Kakagon and the influence of fluctuating water levels on Lake Superior contribute to the wild rice productivity of this wetland.

TAXONOMY

The dynamic taxonomy of the wild rice Genus, *Zizania* is suggested in Table 1a, where authorities have disagreed on the nomenclature of the sub-family and tribe for wild rice. A recent revision of the grass family (Poaceae) has placed *Zizania* in the sub-family Bambusoideae, tribe Oryzeae (Campbell 1985, uncited) and sub-tribe Zizaniinae (Terrell and Robinson 1974). Most treatments recognize four species in the genus *Zizania* (Dore 1969; Warwick and Aiken 1986), two perennial and two annual species. (See Table 1b.) The perennial species occur outside the "wild rice district," one in Asia and the other, an endangered species, in Texas. Both annual species are sympatric; that is, they are both found in the same area, the Upper Midwest. Each species occurs with several varieties. (See Table 1c.) One annual species, *Z. palustris*, can be distinguished from the other, *Z. aquatica*, by its larger edible grain, leathery hulls (lemmas), and hairs on the seed that are restricted to rows over the vascular bundles (Warwick and Aiken 1986; Duvall and Biesboer 1989).

Zizania palustris has two varieties, one I like to call northern wild rice (*Zizania palustris* var. *palustris*), and the other, interior wild rice (*Zizania palustris* var. *interior*). Northern wild rice is distinguished from interior wild rice by its much smaller stature (0.7 to 1.5 m vs. 0.9 to 3.0 m) and smaller leaf width (4 to 14 mm vs. 10 to 30 mm) (Warwick and Aiken 1986). Domestic cultivars used in the paddy industry are thought to be derived from *Z. palustris* var. *interior* (Warwick and Aiken 1986). *Z. palustris* var. *palustris* is the variety found throughout the Kakagon wetland complex, although there are reports that *Z. palustris* var. *interior* was planted near the mouth of the Kakagon River in the late 1930s in an attempt to increase plant yield. I have not been able to positively identify plants that may be of this planting. All discussion of wild rice or northern wild rice throughout this paper will be in reference to *Zizania palustris* var. *palustris*, unless stated otherwise.

PHENOLOGY AND BIOLOGICAL LIFE HISTORY

The following description of the phenology of wild rice relies heavily on repeated visits over six field seasons (1985 through 1990) to several general areas along the Kakagon River. One of these locations is upstream near the Bad River fish hatchery on Little Round River. Wild rice development at this location occurs about 5 to 6 days earlier than at the other location midway down the east fork of Big Round River. (Much farther down river, near the mouth of the Kakagon, wild rice development can be up to two weeks behind these upstream locations in its phenology.) For the description of the phenology presented below, I report the average date of development between these two locations as recorded in 1989. Development is described for wild rice plants at intermediate water depths (about 0.50 m) unless noted otherwise.

Germination/Submerged Leaf/Floating Leaf Stages

Germination of wild rice seed begins immediately at "ice-out," which can vary between early April and early May. For example, in the Kakagon River in 1989, I first observed seeds germinating on April 24. Submerged leaves emerge from the sediment about one week later (May 2). By mid-May, there was a blanket of submerged-leaf plants throughout the riverine habitat of the Kakagon, and floating leaves of wild rice began to appear along the shallow depths. Wild rice development in the Kakagon is earlier than many of the inland lakes of northern Wisconsin, which is directly related to the later "ice-out" dates on the inland lakes. By June 1, most of the wild rice plants in the Kakagon have produced the additional floating leaves, with a few (< 5%) of the shallower water plants beginning to

Table 1. Taxonomic treatments of Zizania.

A) A recent history of subfamily, tribal and subtribal treatments

Authority	Subfamily	Tribe	Subtribe	Genus (#spp)
Hitchcock and Chase (1951)	Festucoideae	Zizanieae	-	*Zizania* (3)
Stebbins and Crampton (1961)	Oryzoideae	Zizanieae	-	*Zizania* (3)
Gould and Shaw (1983)	Oryzoideae	Oryzeae	-	*Zizania* (4)
Campbell (1985)	Bambusoideae	Oryzeae	(3)*	*Zizania* (4)

* Subtribes of Oryzeae (10 Genera, 100 spp.) - (Terrell and Robinson, 1974)

1) Oryziniae- *Oryza, Leersia*
2) Zizaniinae - *Zizania*
3) Luziolinae - *Luziola, Zizaniopsis*

B) Genus Zizania (According to Campbell, 1985 who recognizes 4 species)

A) Perennial (2) - *Z. latifolia* (grisb) Turcz. ex Stapf
Z. texana Hitch.

B) Annual (2) - *Z. aquatica* L.(3 varieties)
Z. palustris L.(2 varieties)
(plus cultivars)

C) Species and varieties (According to Warwick and Aiken 1986)

Z. aquatica		**Z. palustris**	
pistillate lemma papery; sparsely scabrous all over; not used as edible grain		pistillate lemma firm; scabrous only in rows, otherwise lustrous; edible grain	
Large	**Small**	**Large**	**Small**
Z. aquatica L. var. *aquatica*	*Z. aquatica* var. *brevis*	*Z. palustris* L. var. *interior*	*Z. palustris* L. var. *palustris*
	Z. aquatica var. *subbrevis* Boivin		
so. Wis., so. Mi., east coast, south	tidal flats St. Lawrence	w. Canada, n.central U.S.	Mi. UP, no. Wis, ne. Mn, Ont.

show aerial leaves. Natural mortality for wild rice is highest in the submerged leaf stage, with an average of 44% of plants surviving this natural thinning process. The floating leaf stage is also a vulnerable stage; my results show that about 56% of the plants survive the floating stage (Meeker 1993). (See Figure 1.)

Emergent Leaf Stage

By the third week of June, about one-half of the wild rice plants are in an early emergent-leaf stage, varying between 2 and 6 inches above the water's surface. In deeper water, aerial leaf development can be as much as 7 to 14 days delayed. Floating leaves are still present on most of these plants, but many of the earlier submerged leaves have decayed by this time and are not observable.

By June 25, 1989, the primary stems (culms) of the wild rice had begun to elongate up through the protective aerial leaf sheaths. These developing stems were approximately 5 to 25 cm long at this point, still under the water's surface, and only a fraction of the length that they ultimately could achieve (125 to 250 cm). The changing contributions of different structural parts to the total weight of an "average" plant are shown in Figure 2 and are taken from data compiled during the 1988 growing season in the Kakagon Sloughs (Meeker, unpublished data). Prior to stem elongation, an early emergent plant's strength and rigidity is supplied almost exclusively by the turgor provided by the sheaths of the aerial leaves. (Turgor pressure is outward cellular force due to the fact that vacuoles in the cells are saturated with water; this stiffens the cell wall, which cumulatively increases the total plant's strength).

The relative contribution to plant structure by these leaf sheaths decreases with time, suggesting that early plant support is provided in the least "energetic" manner, that is, by cells with high water content, not by cells with increased cellulose content (which would be more costly to the plant). Figure 2 also shows the increasing importance of the more cellulose-rich and costlier stem in providing support after mid-June. It is important to note that the stem elongation takes place *after* an individual plant's floating leaves have reached the water's surface, and presumably these floating leaves transfer a major portion of their accrued photosynthetic gain to rapid stem elongation. This "leaf first, then stem" development is different from many other emergent aquatic plants that develop leaf and stem tissue at the same time. This may be the foremost reason why wild rice, an annual species (relying only on the meager stored resources in its seed), can compete with perennial emergent species that receive much of their early season development from large rhizomatous reserves.

Tillering and Flowering

Tillers (secondary flowering stems) grow out from nodes of the original plant. They are noticeable by mid-June but only by completely uprooting the plant and looking at the start of tiller development from the first node (which is generally under water). By July 15, about 75% of the wild rice stems are in the early flowering stage. Botanically speaking, wild rice is monoecious, in that its sexes are separate but on the same plant. In addition, wild rice flowers protogynously, with the female flowers on each inflorescence borne above and developing before the males. On any individual plant there appears to be little overlap between male and female development, and outcrossing is thought to be the norm (Elliott 1980). Early female flowering is recognized by noting the feathery white stigmas protruding from the protective seed coverings (lemma and palea), and the flowers themselves are just visible sticking up from the protective leaf sheaths. Since the female flowers are small and inconspicuous at this stage, many observers mistakenly believe the male flowers emerge first. Following the ephemeral emergence of the female flowers, the bright yellow male flowers open and disperse their pollen. Pollen viability appears to be negatively correlated with temperature and positively correlated with humidity (Elliott 1980), and fertilization occurs within two hours of pollination (Weir and Dale 1960). Hot, dry days at time of pollen release can hinder fertilization success and reduce later seed set.

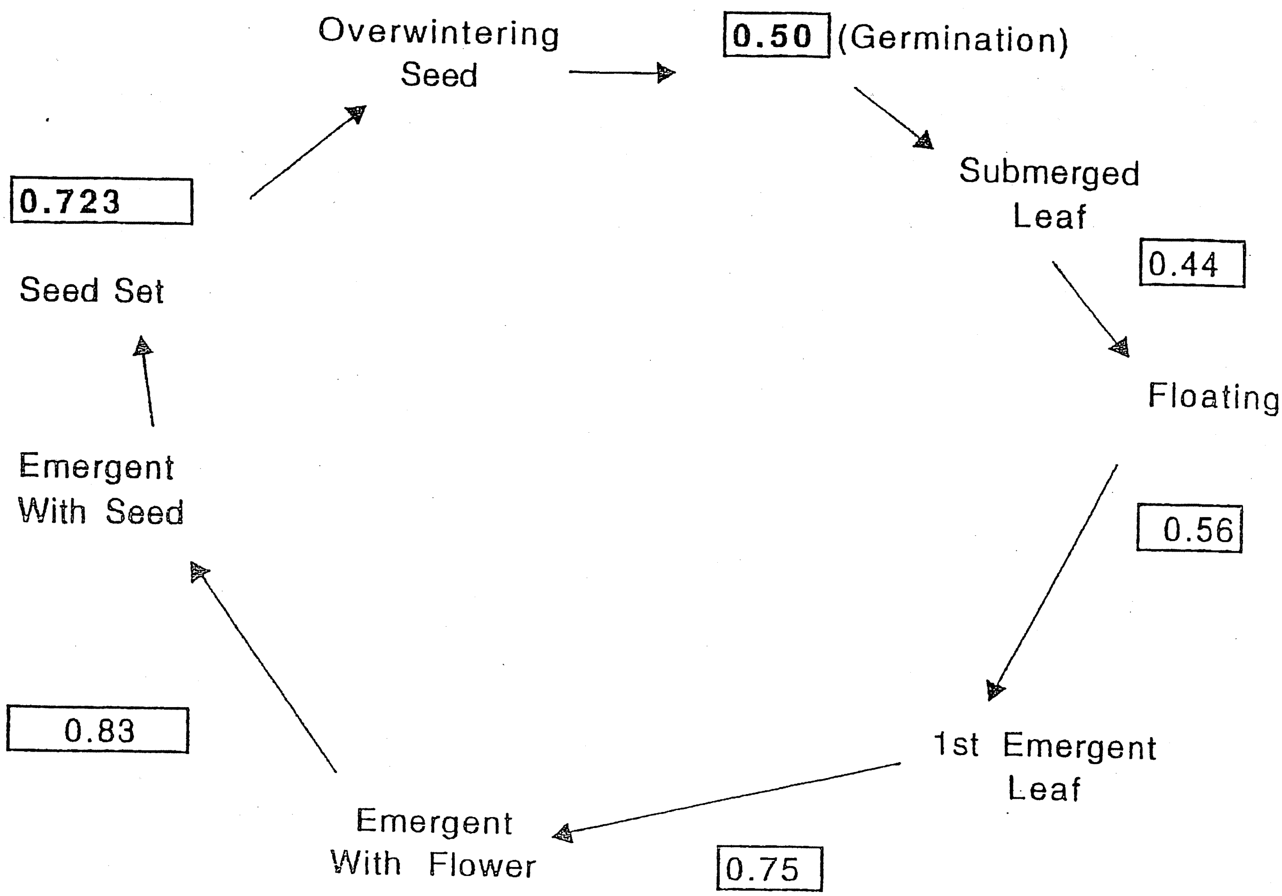


Figure 1. Survivorship for the transition between each of the successive life stages of wild-rice. Numbers in bold-faced type are single estimates, and others are means (n=16).

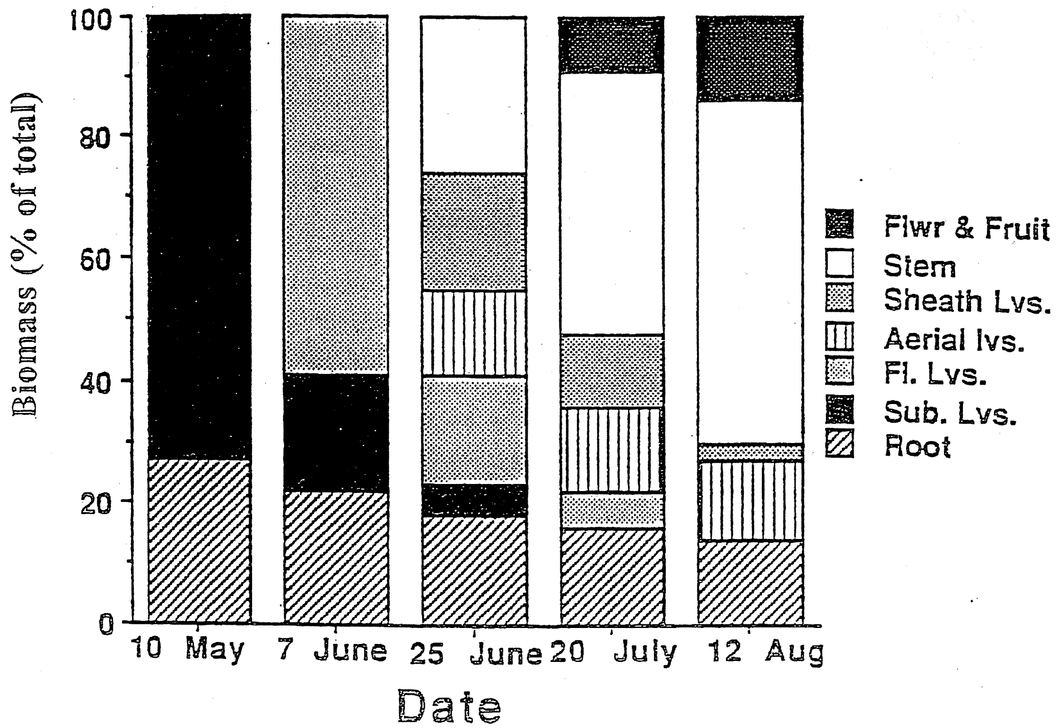


Figure 2. Percent biomass allocated to component parts for representative wild-rice plants sampled over five successive dates, 10 May = submerged-leaf, 7 June = floating-leaf, 25 June = emergent leaf, 20 July = emergent leaf with stem elongation, and 12 August = post-flower and early grain formation. Data are from the Kakagon Slough in 1988.

By mid-July, the tillers are fully emerged and would likely be counted in any estimates of stem density taken at this time. Tillers are 7 to 14 days behind the main stem in their maturity, and, for this reason, contribute to the variability in the final harvest date.

By August 1, the grain on some of the shallow water plants is at the early mature, or "milk" stage, when seed is soft and chewable. Most of the plants are still 2 to 3 weeks behind this early development. The best indication for this early stage of grain formation is the flocking of the black birds that begin feeding on the developing grain (and rice worms, depending on their abundance).

By mid-August, much of the wild rice along the upper stretches of the Kakagon has reached maturity. This is 10 to 14 days prior to maturity in the downstream areas. Hence, the Bad River Natural Resource Department usually opens the ricing season in the Kakagon in two stages. The upper reaches are usually opened by August 15 to 20, with the remaining stands opening one week later. Grain maturity in rice plants is quite noticeable as the plants turn to a buff or straw color as energy is translocated to the developing grain. For an individual wild rice plant, the grain develops and falls (shatters) over a 10- to 14-day period. The research directed toward domestication of wild rice that began in the late 1950s and continued through the 1970s relied upon the discovery of plants that dropped their seed in a more synchronous manner. These semi-"non-shattering" varieties have been the mainstay of the paddy rice industry, now concentrated in Minnesota and California.

Senescence

By mid-September, most of the plants have dropped their seed. Upon dropping, the individual grains readily sink into the water and work their way into soft sediment near the parent plant (Dore 1969). It has been suggested that the retrorse barbs along the long awn of each seed are an adaptation for self burial, where the barbs resist dislodging (Bayly 1983).

Most of the leaves and leaf sheaths have disintegrated by mid-September, though some of the stalks remain erect for a long time. I have seen a number of stalks frozen in the ice, and a small percentage are still erect within the water column at "ice-out" the following season.

Wild rice seed lies dormant in the sediment until the following spring. Typically, about half of one year's seed cohort germinates the next spring (Atkins et al. 1987; Meeker 1993) due to a second dormancy and non-viable seed. Most viable seed germinates in the first year, but about 10% of this seed can remain dormant for up to five years (Meeker, personal communication with P. F. Lee). Both mechanical (pericarp resistance and impermeability) and hormonal conditions (increased concentrations of inhibitory hormones in hulls and pericarp of fresh seed) appear to regulate dormancy in wild rice (Caldwell et al. 1978). The extended dormancy in wild rice is apparently an adaptation allowing the species to survive temporary unfavorable conditions or total crop failures in any given year.

ECOLOGY

Variability in Wild Rice Abundance

Historically, wild rice is known to be quite variable in abundance both temporally and spatially (Moyle 1944; Rogosin 1951; David, this volume). In this context, I refer to temporal variability as the year-to-year change in abundance (i.e., yield, stem density, and acreage) of wild rice at the same location. Spatial variability has two components, including the variability in wild rice over time between any two locations and the variability between any two locations in a given season.

A number of principal ecological factors have been suggested by the literature as being important in the ecology of wild rice and, hence, influencing the variability in wild rice abundance. These factors operate at different scales, and their importance in a riverine environment is, in turn, influenced by differences in channel morphometry and current velocity. The literature does not specifically address the ecology of wild rice in riverine environments; therefore, the relationship between different physical parameters along a river and those known to influence wild rice in the literature are emphasized in the following sections.

Water Chemistry

Studies have investigated the geographic distribution of wild rice with attention given to the possible differences in water chemistry from site to site (Archibold and Weichel 1986; Chambliss 1922). Moyle (1944) suggested that wild rice does not grow in water with greater than 50 ppm sulphate, limiting the westward distribution of wild rice in Minnesota. Other characteristics of water chemistry do not appear to be limiting, as wild rice grows over wide ranges of alkalinity, pH, iron, and even salinity (Rogosin 1951). Much of this early information is still referred to in the literature today (Fannucchi et al. 1986), attesting to its accuracy. However, knowing the variability of wild rice, it also attests to the lack of utility of such measurements in predicting wild rice distributions on a local scale. Large-scale testing of water chemistry in the Kakagon Sloughs, including PH and specific conductivity (Meeker, unpublished data), has indicated some variability over a wide area but does not appear to be an important factor in wild rice distribution in this system.

Disturbance

Disturbance has also been suggested as important in rice ecology (Rogosin 1951). Dore (1969) relates the classic story of a pilot who, while flying, frightened a moose that was feeding in a thick stand of perennial aquatic vegetation. The moose ran off, cutting a wide swath in the perennial vegetation that was colonized in the following year by a dense stand of wild rice. Similar "openings" of the habitat can result from ice scour (Dore 1969). Burial of wild rice in highly flocculant sediment is offered by Lee (1986b) as a key problem in attempting to colonize lakes presently void of wild rice.

I investigated the effects of both sediment and thatch burial on wild rice emergence in the Kakagon by manipulating levels of thatch accumulation and sediment thickness by planting wild rice in a number of "cribs" at contrasting levels of thatch and sediment burial.

Seed burial under 8 cm of sediment resulted in almost a complete absence of emerging seedlings across both depths, whereas plants developing under moderate and no sediment burial showed about a 20 to 30% survival (Meeker 1993). (See Figure 3.) Thatch burial also reduced emergence, but not as dramatically as sediment burial.

Other disturbances in wetlands include annual water level fluctuations, which are discussed in the Water Depth section. It should be noted here, however, that in Great Lakes wetlands, water level changes are necessary to maintain wetland viability. They are thought to be necessary for long-term wild rice productivity in the Kakagon Sloughs, as discussed in the Competition section (Meeker 1993).

Water Depth

Much of the year-to-year variability in wild rice abundance has been correlated with the "within season" fluctuations in water levels (Thomas and Stewart 1969; Stephenson and Lee 1987; Lee 1986b; Pip and Stepaniuk 1988). Rogosin (1951) suggests that increases in water level lower light penetration, which then directly reduces the plants' capability to reach the surface and begin photosynthesizing as a floating leaf and emergent plant. Much anecdotal evidence suggests that rapid water level changes can destroy whole crops by uprooting vulnerable floating leaf plants or by drowning plants after the floating leaf stage (Fannucchi et al. 1986; Lee 1986b).

Water level gradients in both rivers and lakes most certainly influence wild rice abundances (Thomas and Stewart 1969; Dore 1969), creating increased spatial variability. Oelke et al. (1982) suggests, for example, that the maximum depth for the growth of paddy wild rice is about 36 inches (0.91 m), whereas, others (Fannucchi et al. 1986; Dore 1969)

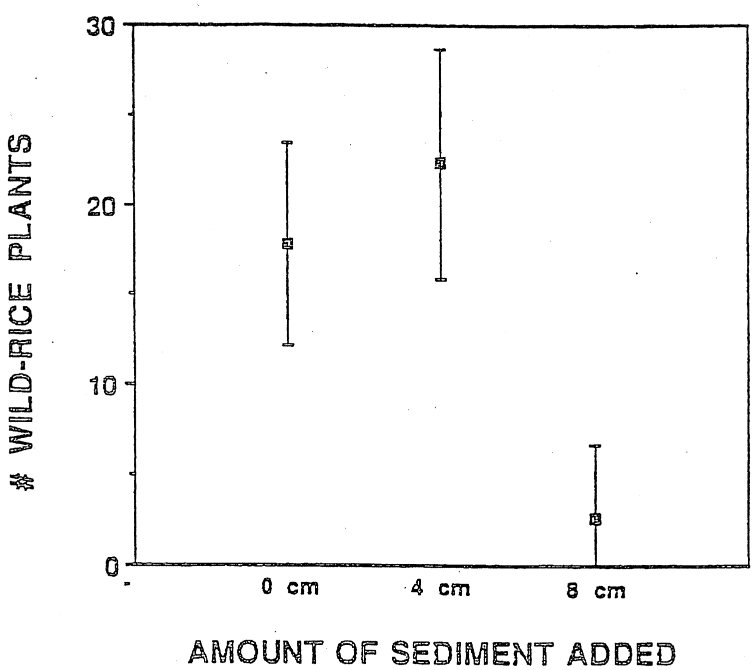


Figure 3. Final number of wild-rice plants remaining from a cohort of 100 wild-rice seeds planted in experimental cribs under 3 levels of sedimentation. Each point represents a mean over 2 depths (n=4). Errors indicate 95% confidence intervals.

report the range of depths for wild rice to be between 6 and 30 inches (0.15 and 0.76 m). I found that the optimal depths for wild rice development fell between 13 and 45 inches (0.35 and 1.1 m) (Meeker 1993), but this range was also much influenced by the location along the riverine habitat (channel morphometry, discussed below). Curiously, it should be noted that southern wild rice (*Zizania aquatica*) is able to flourish in presumably nutrient-rich tidal environments with considerable diurnal changes in water level (Chambliss 1922; Ferren and Good 1977).

Substrate Quality/Nutrient Analysis

Substrate differences and nutrient availability have also been suggested as a major determining factor in wild rice spatial variability (Day and Lee 1989; Lee and Stewart 1983). Fannucchi and others (1986) summarize the literature when they suggest that the ideal substrate for wild rice is about 18 inches (about 45 cm) of soft muck sediment (Dore 1969). Recent research has concentrated on efforts to determine the suitability of specific lake environments for establishing wild rice stands. In this context, environmental regions within lakes have been correlated to an extent with wild rice densities (Lee 1986a). In one case, wild rice was found to be in positive association with a specific pondweed species (*Potamogeton robbinsii*), which, it was suggested, had ameliorated the site to the benefit of wild rice (Day and Lee 1989).

Nutrient analysis of wild rice in agricultural situations (rice paddies) has been studied relative to fertilizer treatments (Grava and Raisanen 1978), but less so in natural settings. In one example of a natural stand nutrient study, Lee and Stewart (1983) were not able to establish good correlations between nutrient uptake and nutrient concentrations in the sediment. Barko and Smart (1986) have suggested that, in general, attempts to relate aquatic plant productivity to sediment nutrients on a mass basis may be more meaningful. They have shown, for example, decreased productivity in submergent aquatic species in highly organic soils (less dense sediment) and suggest that greater diffusion distances required for nutrient uptake in flocculent settings as possible reasons for these findings. These findings may also explain the general lack of success in restoration of wild rice in more flocculent sediment (David, this volume).

Hydrology and Sediment Flux (as It Is Affected by Channel Morphometry)

Another abiotic factor that has been suggested in the literature as being important in influencing wild rice abundance is related to the hydrology of the wild rice habitat. Much of the traditional literature (Jenks 1901; Moyle 1944; Rogosin 1951; Dore 1969) suggests that an ideal habitat for wild rice consists of "slowly moving waters," suggesting that an influx of sediment may be important in creating optimal wild rice habitat (Dore 1969). Lake habitat, without a source of nutrient-rich sediment, may be expected to deteriorate over time. Indeed, it has been noted that newly established stands lose vigor over time (Peden 1982; Keenan and Lee 1988), suggesting that wild rice may mine the substrate much like many annual agriculture crops.

At any specific location in the Kakagon, both channel morphometry and water depth greatly influence the physical environment that developing wild rice plants encounter after germination. In addition, the wild rice plants themselves modify the local environment and greatly influence the sedimentation regime. Seasonal sedimentation rate was seen to vary among four locations that differed in their channel morphometry. Sedimentation was greater in riverine areas compared to the backwater and also differed among the growth stages of wild rice (Meeker 1996). The data suggest that in many locations along the river, the early growth stages of wild rice (the submersed and floating leaf stages) act as filters and trap sediment that then provides the nutrients necessary for the later demanding stages of stem elongation and grain development. Annual cycles of maximum wild rice stand growth immediately follow the import of sediments and their associated nutrients, as suggested by changes in growth allocations of wild rice over time. (See Figure 2.)

Wild rice productivity was also directly related to differing channel morphometry and seasonal sedimentation rate in the Kakagon Sloughs. (See Figure 4.) Peak stand biomass (>400gm $-m^2$ dry weight) and seed yield (> 2000 m^2) were found at intermediate depths in areas with high annual sediment input. In general, there was less biomass and seed yield in backwater areas with little annual sediment input. Wild rice stem density varied greatly among sites and locations along the depth profiles. This was true even for areas with similar total biomass, as some stands had "many, smaller plants" while others had "fewer, larger plants." Dense wild rice populations having many, small plants may act as deterrents for colonization by competing species, suggesting techniques for restoring riverine wild rice.

Competition

Although the literature makes abundant reference to the fact that wild rice faces stiff competition from other aquatic macrophytes (Dore 1969; Rogosin 1951; Lee 1986b; Fannucchi et al. 1986), most of the discussions are post hoc. Observations between 1986 and 1987 in the Kakagon Sloughs suggest individual species' phenology may play important roles in long-term competitive interactions. Yellow water lily (*Nuphar variagata)* for example, sets its fully expanded leaves on the water's surface 7 to 10 days prior to wild rice reaching the floating leaf stage, suggesting a significant interspecific competition for light. Other floating leaf taxa such as several species of *Potamogeton* do not appear to interfere with wild rice in light gathering at an early stage at water depths less than 1 m but do appear to occupy areas deeper than 1 m in wild rice areas. Finally, submerged species have been suggested to be major competitors with wild rice (Lee 1986b). In the Kakagon Sloughs, however, these species appear to occupy areas that wild rice is precluded from due to excess thatch or sediment build-up (Meeker 1993).

In the Kakagon, direct competition experiments were conducted between wild rice and yellow water lily by both planting rice under different levels of lily cover and simulating lily cover over existing wild rice stands. The results indicate that competition with water lily did not affect emergence (as was expected), yet reduced final survival, especially at greater water depths. Within each level of lily competition, wild rice survival was less in deeper water. (See Figure 5.) The competition experiment data suggest that there may be a threshold weight for individual plants, below which wild rice mortality is increased.

In addition to direct competition studies, I monitored for presumed competitive interactions by monitoring for changes in percent cover of wild rice and other macrophytes in response to more than four years of water level fluctuations in Lake Superior. This period of study followed a water level drawdown of about 0.50 m between 1986 (a high water year) and 1988 (a low water year) (Wilcox et al. 1992). Data demonstrates that drawdown years, like 1988, are important in maintaining long-term wild rice abundances by allowing this annual species to rapidly re-colonize areas that were too deep for most aquatic species during high water years. (See Figure 6.) It is suggested that regulation of water levels in the Lake Superior basin toward a more stabile water level regime would be very damaging for this productive wild rice wetland.

SUMMARY

Although the above mentioned factors are recognized as important in influencing the abundance and distribution of wild rice, the literature does not specifically address the ecology of wild rice in riverine environments. In river habitats, a number of the factors listed above (e.g., substrate quality, disturbance, and competition) are influenced by differences in channel morphometry and current velocity.

In general, wild rice is well adapted to the riverine environs. As an annual plant competing with perennials, it benefits from moving waters through both the seasonal pulse of sedimentation and the scouring action or opening of habitat. In addition, annual water level fluctuations appear to favor wild

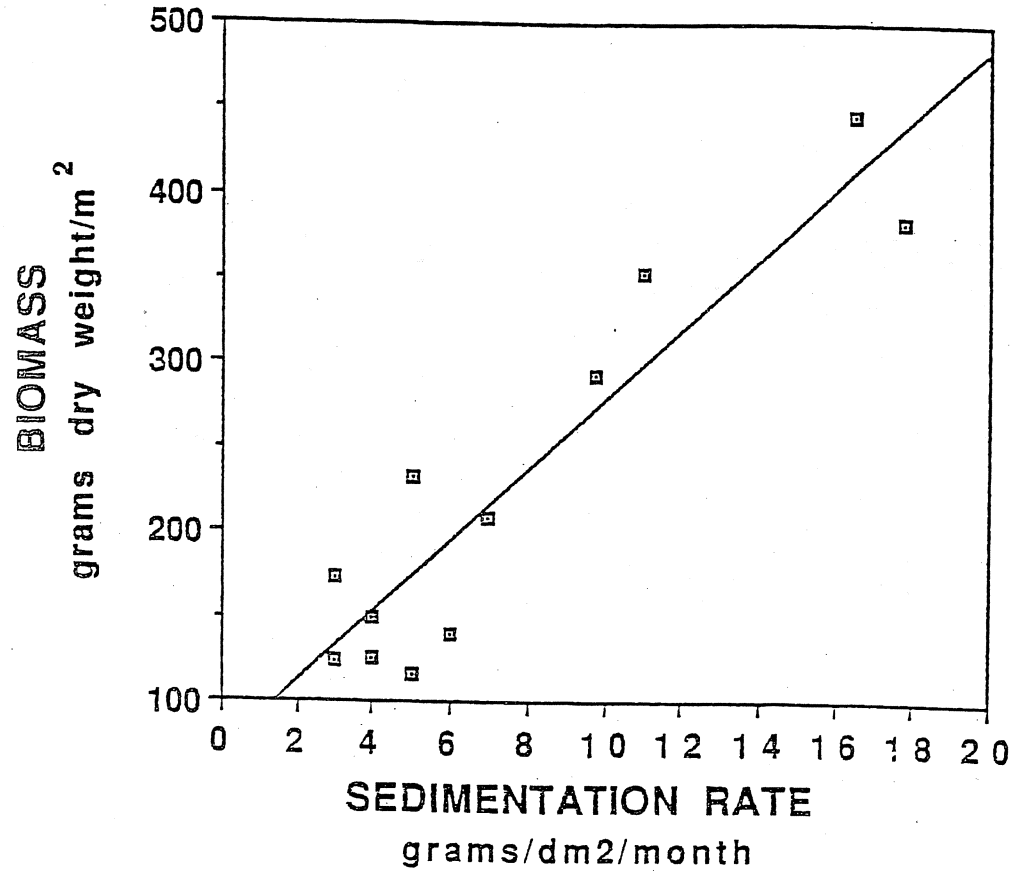


Figure 4. Final biomass (g dry-wt m^{-2}) on August 10, 1989 vs. sedimentation rates (g dm^{-2} mo^{-1}) during the 1989 field season. Regression equation is $y = 69.45 + 20.20x$, $R^2 = 0.86$, $p>.05$.

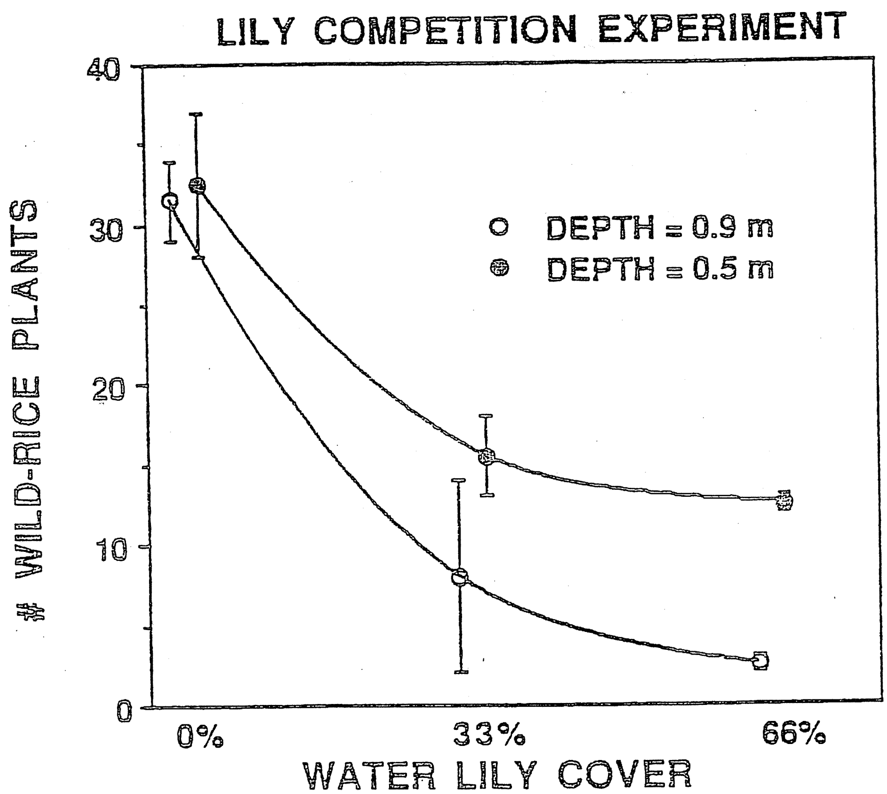


Figure 5. Final number of wild rice plants remaining from a cohort of 100 wild rice seeds planted in experimental cribs under three levels of competition. Planting occurred at 2 depths, 0.9 m (open circles) and 0.5 m (closed circles). Error bars indicate the range of 2 replicates for each point.

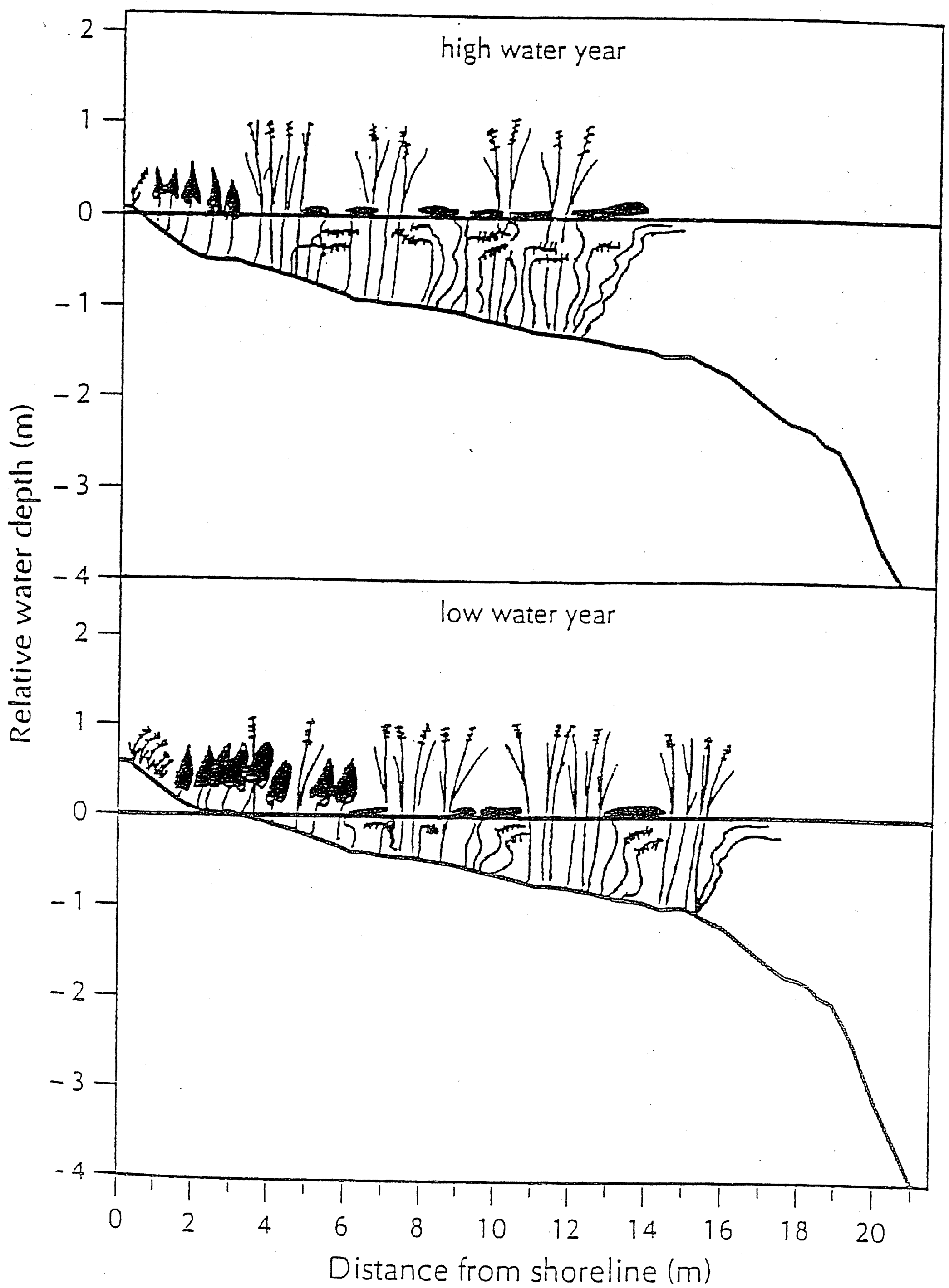


Figure 6. Schematic of a dynamic pattern of macrophyte distribution in a representative profile of a Kakagon River transect depicting changes from (a) high water year to (b) low water year. Drawing is based on actual cover data for 1986 (a) and 1988 (b).

rice productivity in the long term. In contrast to the generally accepted view that stabile water favors wild rice, flooding events, although damaging to wild rice in a given season, act to set back perennial competition and offer a more open habitat for wild rice re-colonization during the subsequent drawdown years. Restoration and management that recognizes these natural processes will likely be sustainable.

REFERENCES

Archibold OW, Weichel BJ. 1986. Variation in wild rice (*Zizania palustris*) stands across northern Saskatchewan. Can Bot 64:1204-11.

Atkins TA, Thomas AG, Stewart JM. 1987. The germination of wild rice in response to diurnally fluctuating temperatures and after-ripening period. Aquat Bot 29:245-59.

Barko JW, Smart RM. 1986. Sediment-related mechanisms of growth limitation in submersed macrophytes. Ecology 67:1328-40.

Bayly IL. 1983. The caryopsis as a support organ for germinating wild rice, *Zizania aquatica*. Can Field-Naturalist 98(3):369-70.

Caldwell VB, Oelke EA, Elliott WA. 1978. Seed dormancy mechanisms in wild rice (*Zizania aquatica*). Agron J.70(6):481-4.

Chambliss CE. 1922. Wild rice. U. S. Dept Ag Circ 229, 16pp.

Day WR, Lee PF. 1989. Ecological relationships of wild rice, *Zizania aquatica*. 8. Classification of sediments. Can J Bot 67:1381-6.

Dore WG. 1969. Wild rice. Canada Dept Ag Res Publ 1393:1-84.

Duvall MR, Biesboer DD. 1989. Comparisons of electrophoretic seed protein profiles among North American populations of *Zizania*. Biochem Syst Ecol 17(1):39-43.

Elliott WA. 1980. Wild rice (*Zizania aquatica*). In: Fehr WA and Hadley HH eds. Hybridization of crop plants. Crop Science Society of America, pp. 721-31.

Fannucchi GT, Fannucchi WM, and Craven S. 1986. The ecology of wild rice. Univ Wis Ag Ext Service.

Ferren RR, Good RE. 1977. Habitat, morphology and phenology of southern wild rice (*Zizania aquatica*) from the Wading River in New Jersey. Bull Torrey Bot Club 104:392-6.

Gould FW, Shaw RB. 1983. Grass systematics. College Station: Texas A&M Univ Press.

Grava J, Raisanen KA. 1978. Growth and nutrient accumulation and distribution in wild rice, Agron J 70(6):1077-81.

Hitchcock AS, Chase A. 1951. Manual of the grasses of the United States, 2nd ed. U. S. Dept Ag Misc Publ No 200.

Jenks AE. 1901. The wild rice gatherers of the upper lakes: a study in American primitive economics. Washington: Government Printing Office.

Keenan TJ, Lee PF. 1988. Ecological relationships of wild rice, *Zizania aquatica*. 7. Sediment nutrient depletion following introduction of wild rice to a shallow boreal lake. Can J Bot 66:236-41.

Lee PF, Stewart JM. 1983. Ecological relationships of wild rice, *Zizania aquatica*. 2. Sediment-plant tissue nutrient relationships. Can J Bot 61:1775-84.

Lee PF. 1986a. Ecological relationships of wild rice, *Zizania aquatica*. 4. Environmental regions within a wild rice lake. Can J Bot 64(9):2037-44.

Lee PF. 1986b. Summary report: the aquaculture of wild rice. In: Proceedings from Wild Rice, the Natural Crop Conference. May 13-15, 1986; Minaki, Ontario.

Meeker JE. 1993. The ecology of "wild" wild rice (*Zizania palustris* var. *palustris*) in the Kakagon Sloughs, a riverine wetland on Lake Superior [PhD dissertation]. Univ of Wis-Madison.

Meeker JE. 1996. Wild rice and sedimentation processes in a Lake Superior coastal wetland. Wetlands 16(2):219-31.

Moyle JB. 1944. Some chemical factors influencing the distribution of aquatic plants in Minnesota. Am Midl Nat 34:402-20.

Oelke EA, Grava J, Noetzel D, Barron D, Percich J, Schertz C, Strait J, Stucker R. 1982. Wild rice production in Minnesota. Extension Bulletin 464. Univ of Minn, Ag Ext Service.

Peden DG. 1982. Factors associated with growth of wild rice in northern Saskatchewan. Arctic 35(2):307-11.

Pip E, Stepaniuk J. 1988. The effect of flooding on wild rice, *Zizania aquatica* L. Aquat Bot 32:283-90.

Rogosin A. 1951. An ecological history of wild rice. Minn Dept of Conservation, Div Game and Fish, Sect Res Plan.

Stebbins GL, Crampton B. 1961. A suggested revision of the grass genera of temperate North America. Recent Advances in Bot 1:133-45.

Stephenson S, Lee P. 1987. Ecological relationships of wild rice, *Zizania aquatica*. 6. The effects of increases in water depth on vegetative and reproductive production. Can J Bot 65:2128-32.

Terrell EE, Robinson H. 1974. Luziolinae, a new subtribe of Oryzoid grasses. Bull Torrey Bot Club 101:235-45.

Thomas AG, Stewart JM. 1969. The effect of different water depths on the growth of wild rice. Can J Bot 47:1525-31.

Warwick SI, Aiken SG. 1986. Electrophoretic evidence for the recognition of two species in annual wild rice (*Zizania*, Poaceae). Syst Bot 11(3):464-73.

Weir CE, Dale HM. 1960. Developmental study of wild rice, *Zizania aquatica* L. Can J Bot 38:719-39.

Wilcox DA, Meeker JE, Elias JE. 1992. Impacts of water-level regulation on wetlands of the Great Lakes. Draft Report to the International Joint Commission's Water Level Reference Study Board.

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